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WHITHER RURAL MEDICINE?

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Pesticide Residues in the Blood of Migrant Field Workers in Relation to Occupational Exposure

*K. R. Long**

Occupational exposure of human beings to pesticides resulting in the accumulation of their residues in body fluids and tissues is a growing public health concern. An opportunity to study such a relationship was provided by a group of Mexican-American migrant field workers in southeastern Iowa during the summer of 1968. The group was composed of approximately 700 field workers and 450 family dependents. The migrant field workers were employed to cultivate and harvest crops by cucumber and tomato farmers whose

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SLIDE 3

COMPARISON OF MEAN CHLORINATED HYDROCARBON PESTICIDE BLOOD RESIDUE VALUES FOR MALE AND FEMALE MIGRANTS

	Number of Subjects	*Pesticide Residue Values*		*Standard Deviation*	*Significance*
Male	12	p,p'DDE	57.0 ppb	33.5 ppb	p<0.05
Female	46		37.6	22.9	
Male	12	Total Chlorinated Hydrocarbon	93.8	46.4	p<0.01
Female	46		47.9	29.2	

SLIDE 4

COMPARISON OF MEAN CHLORINATED HYDROCARBON PESTICIDE BLOOD RESIDUE VALUES FOR BOTH MALE AND FEMALE MIGRANTS GROUPED BY AGE

Age Group	*Number Subjects*	*Mean Pesticide Values*		*Standard Deviation*	*Significance*
Below 20	13	p,p'DDE	31.9 ppb	11.7 ppb	p<0.05
Above 40	45		38.6	15.8	
Below 20	13	Total Chlorinated Hydrocarbon	49.5	27.8	p<0.01
Above 40	45		75.5	34.1	
Below 35	35	p,p'DDE	36.9	22.6	p>0.10
Above 35	23		48.7	30.3	
Below 35	35	Total Chlorinated Hydrocarbon	48.1	33.1	p<0.05
Above 35	23		71.7	41.0	
21-30	16	p,p'DDE	40.6	28.7	p<0.80
31-40	15		43.3	30.7	
21-30	16	Total Chlorinated Hydrocarbon	54.5	43.7	p<0.70
31-40	15		59.9	42.9	

acreage was under contract to a large food processing firm. The tasks were accomplished primarily by hand labor which brings the field worker into intimate contact with an environment where large amounts of pesticides are used. The peak migrant population during 1968 was between August 1 and October 1, during which time the study was conducted.

A once-a-week family clinic for nonemergency health problems of the migrant families provided an excellent opportunity to obtain blood samples from the adults and from children when possible. Occupation, past medical history and medical examination data were also obtained. The blood samples were analyzed for chlorinated hydrocarbon pesticide residues by the method of Dale, Curley and Cueto[1] employing a Micro-Tek 220 gas chromatograph equipped with a 130 micro-Curie electron capture tritium detector.

The mean values of p,p'DDE and total chlorinated hydrocarbon pesticide blood residues obtained for all male migrants tested are shown on Slide 1 by age group. The number of subjects in each age group is noted by the number immediately above the bar for that group. The number of male subjects examined was less than the number of females. This was attributed to what appeared to be a general reluctance on the part of the migrant male to attend the voluntary weekly clinics. It may be observed from this slide that there is a gradual increase in the average amounts of p,p'DDE and total chlorinated hydrocarbon pesticide residues from age 11 to age 40 at which time the values appear to decline. These data must be viewed with some caution since the number in each group is quite small.

Similarly the mean values of p,p'DDE and total chlorinated hydrocarbon pesticide blood values obtained for all female migrants tested are shown on Slide 2 by age group. Contrary to the findings for the male migrants, one may observe that there is very little change in the

SLIDE 7

COMPARISON OF MEAN CHLORINATED HYDROCARBON PESTICIDE BLOOD RESIDUE VALUES FOR MIGRANT HOUSEWIVES AND FEMALE FIELD WORKERS

	Number of Subjects	*Pesticide Residue Values*		*Standard Deviation*	*Significance*
Housewives	17	p,p'DDE	33.5 ppb	26.2 ppb	p<0.40
Field workers	29		39.9	20.9	
Housewives	17	Total Chlorinated Hydrocarbon	39.1	30.6	p<0.10
Field workers	29		53.1	27.5	

SLIDE 8

COMPARISON OF MEAN CHLORINATED HYDROCARBON PESTICIDE BLOOD RESIDUE VALUES FOR MIGRANT MALE AND FEMALE FIELD WORKERS

	Number of Subjects	*Pesticide Residue Values*		*Standard Deviation*	*Significance*
Male	12	p,p'DDE	57.0 ppb	33.5 ppb	p<0.10
Female	29		39.9	20.9	
Male	12	Total Chlorinated Hydrocarbon	93.8	46.4	p<0.01
Female	29		53.1	27.5	

mean values of either p,p'DDE or total chlorinated hydrocarbon pesticide residues until age 40 at which time these values appear to increase. As before, the number of subjects in each age group is noted by the number immediately above the bar for that particular group. Since there appeared to be differences between the residue values for males and females, a comparison was made utilizing the test as shown in Slide 3. A statistically significant difference was obtained at $p < 0.05$ when p,p'DDE values were compared and a significant difference at a probability level of $p < 0.01$ when total chlorinated hydrocarbon residue values were compared. The differences observed with the data for male and female migrants as presented on Slides 1 and 2 were combined and compared statistically by the p,p'DDE residues between those under 20 and those over 40 years of age was significantly different at a probability level of $p < 0.05$. When the mean values for total chlorinated hydrocarbon residues were compared for the same groups a statistically significant difference at $p < 0.01$ was obtained. If one divides the subjects into two groups, one under 35 and one over 35 years of age, and compares the pesticide residue values, one obtains no statistically significant difference for p,p'DDE residue values but does obtain a statistical significance of $p < 0.05$ when total chlorinated hydrocarbon pesticide residue values are compared. One obtains no statistically significant difference when one compares the 21-30-year age group with the 31-40 group. These comparisons point out that the primary differences in pesticide residue values exist between the young migrants, less than 20 years of age, and the older migrants, those over 40 years of age. From these data one may surmise that older migrants have higher blood chlorinated hydrocarbon residue values because of greater exposure particularly if one assumes that the subject absorbs additional pesticide during a subsequent season before he eliminates those residues absorbed priorly.

When the migrant families were enrolled in the clinics, they were asked their primary occupation. The males were uniformly classed as field workers while the females fell into two groups, field workers and housewives. The chlorinated hydrocarbon pesticide residues for the migrant housewives are shown in Slide 5 by age group. It may be noted that the residue pattern is quite similar to that obtained for the grouped data of all females. The residue values for female field workers are shown on Slide 6. Here it may be observed that the residue pattern is similar to that found for the male field workers which is quite dif-

ferent from the pattern of the migrant housewives. It will be observed that the peak residue values for the female field workers occur in the 21-30-year-old group rather than in the 30-40 year group as shown previously for the male migrants.

With these groupings in mind the residue values for female field workers were compared by the test to those of the migrant housewives as shown on Slide 7. The difference in p,p'DDE residue levels and total chlorinated hydrocarbon between the two groups was not significant at the 5% level. A similar comparison was made between male migrants and female field workers as shown on Slide 8. The magnitude of the residue values between these groups was not statistically significant at the 5% level for p,p'DDE but was significantly different for total chlorinated hydrocarbon pesticide at a significant level of $p < 0.01$.

In presenting these data the residue values for p,p'DDE, a metabolite of p,p'DDE, were singled out since this pesticide residue commonly occurred in the blood of all the subjects examined. The substances included in the category "total chlorinated hydrocarbon residues" included p,p'DDE, p,p'DDD, benzene hexachloride and heptachlor epoxide.

Epidemiological investigation revealed that DDT, DDD, heptachlor and aldrin were chlorinated hydrocarbon pesticides used in the area where the migrants were working. The conditions under which this study was conducted do not permit one to examine the relationship between the pesticides used by the farmers in the area and the residue values found in the bloods of the migrants who were employed by them. A factor having a significant bearing upon these findings is the seasonal manner in which the migrants are employed. The migrants coming to Iowa are from the Lower Rio Grande Valley of Texas where they reside and work during the winter months in the truck gardening activities of this area. No information is available on the chlorinated hydrocarbon pesticides to which they may have been exposed during this time.

There were a number of ailments diagnosed during the family clinics among them upper respiratory illnesses, ear infections, skin diseases and gastrointestinal disorders. None of these appeared to be related to exposure to pesticides nor was any disease state isolated to any particular occupational group.

In summary the data presented show significant differences in chlorinated hydrocarbon pesticide residue values between male and female migrant workers and between those under 20 and those over 40 years of age. These differences are believed in part due to greater exposure by the older subjects and the males to a work environment where pesticides are prevalent.

This study was supported by Contract No. PH 86-66-52, Community Studies Division, Food and Drug Administration, Consumer Protection and Environmental Health Service, Public Health Service, Department Health Education and Welfare.

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Discussion

M. Wassermann (Israel): You have obtained data demonstrating that the females have a lower concentration than the males. The second aspect, which is very interesting to me, is that above the age of 40 years or 35 years the concentration is higher and that below the age of 20 years the concentration is lower. In fact, such a condition occurs also in the storage of organochlorine insecticides. I am very pleased with the results of your study, because I am discussing with several organizations. We can see that it will be possible in the future to evaluate only the blood concentration and it will be unnecessary to call for the samples of body tissues.

The second fact about which I would like to have an explanation is as follows. In the paper you have published in association with industrial hygiene, you have shown there is no difference between the concentration and occupationally exposed people and nonexposed people as far as the concentration of organochlorine insecticides is concerned. But at the same time, you have shown that as for people with a high exposure to insecticides there are differences in hematocrit, hemoglobin, bilirubin and prothrombin time. How do you explain this aspect?

K.R. Long (USA): First of all, I would like to comment with respect to residue by use in the blood as compared to the residue in the tissues. There appears to be some

sort of ratio set up between the tissue levels and the residue value that occur in the blood of the human being. The current work being done by the pesticide program includes monitoring members of the general population both with respect to residues in the fat tissue as well as residues in the blood getting a pair of blood and fat samples from each subject. This datum is not complete at the moment. So I am not capable of giving you what the ratio might be.

I might add in response to an earlier comment by, I believe Prof. Wassermann, that under certain stress conditions you will find that the blood value of the residues will elevate with respect to chlorinated hydrocarbon. These conditions can be chronic disease conditions or also conditions of stress. These things have to be borne out or have to be studied in greater detail with respect to what the blood residue values actually mean when we are trying to put them together with respect to illness and health.

With respect to the second part of your question, Prof. Wassermann, that has to do with the paper that is published in the *American Industrial Hygiene Journal*. This was a part of the prospective epidemiology study of a group of farmers within a defined area in the state of Iowa. The values reported for chlorinated hydrocarbon were primarily those values for DDE. Now, there appears to be some discrepancy with respect to this, because there were, in association with this, differences noted in the clinical biochemistry of these individuals.

We have an attempt to see if a relationship could be established between the residues and also the materials used by the individuals and their clinical biochemistry to get some statistical analyses and find certain significant results. It is extremely difficult, as brought out by Dr. Doreman and his co-workers, to really evaluate the effect of exposure as measured by the blood residue levels. There are many, many variables, and we do not completely understand with respect to the relationship of the blood residue levels, exposure and subsequent disease.

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25. *Concentration of DDT in Brain and Other Tissues in Relation to Symptomatology.* WAYLAND J. HAYES, JR. and WILLIAM E. DALE, Toxicology Branch, Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia.

Following repeated doses of DDT at a dietary rate of 200 ppm, rats tolerated without significant inconvenience storage of the compound in the lipids of adipose tissue in concentrations averaging $>$ 500 ppm in males and $>$ 1400 in females. Under these conditions, the concentration of DDT in the brain lipids did not exceed 280 ppm and averaged $<$ 170 ppm. Animals that died following repeated doses showed concentrations of DDT in brain lipid ranging from 205 to 985 and averaging 595 ppm in males and 767 ppm in females.

Similar concentrations of DDT (524–848 ppm in males) were found in rats that died following a single dose of the compound although the DDT in lipids from fat averaged only 361 ppm with a maximum of 424 ppm. The degree of illness, ranging from mild tremor to death, corresponded with the concentration of DDT in the brain and to a lesser degree with the concentration in the plasma, liver, and kidneys. The degree of illness did not correspond to the concentration in the fat. Thus, chemical confirmation of poisoning by DDT cannot be based on the concentration of DDT in body fat unless the dosage schedule or the concentration in other tissues, or both, can be determined.

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Treatment of Severe Parathion Poisoning with 2-Pyridine Aldoxine Methiodide (2-PAM)

Report of a Case

ARNOLD J. FUNCKES, M.D., Ph.D., Phoenix, Ariz.

The use of organic phosphorus pesticides (parathion, malathion, Diazinon, etc.) has increased greatly in the last 10 years. As helpful as these materials are to agriculture, problems have resulted from the use of some of them that have appreciable mammalian toxicity and that have been the cause of numerous human poisonings.

Organic phosphorus compounds exert their toxic effects by inhibiting cholinesterase activity, thereby allowing the accumulation of acetylcholine. In addition to artificial respiration and other supportive measures, the accepted method of treating these poison cases is with atropine. This form of therapy has saved many lives. Unfortunately, atropine treatment is not specific and only partially blocks the effect of accumulated acetylcholine.

Wilson and others[1,2] have introduced the use of 2-pyridine aldoxime methiodide (2-PAM) as a specific reactivator of alkylphosphate-inhibited cholinesterase. 2-PAM has reactivated successfully the inhibited acetylcholinesterase in vitro, and animals injected with lethal doses of alkylphosphates were spared by 2-PAM injection.[2,3] 2-PAM has been shown to have relatively low toxicity in animals. These toxic effects are manifested only by doses in excess of that required to regenerate the enzyme. Experience with the use of 2-PAM in human poison cases is meager.[4,5]

The degree of physiological effect of parathion is reflected by cholinesterase inhibition. The degree of exposure to parathion is reflected by the amount of urinary paranitrophenol excreted[6] and by the amount of parathion that can be removed from the patient's body by alcohol, even in spite of numerous washings with soap and water.

In the following case, blood cholinesterase activities, urinary paranitrophenol values, and measurement of parathion in skin washes were obtained following the administration of 2-PAM.

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From the Technology Branch, Communicable Disease Center, Public Health Service, U.S. Department of Health, Education, and Welfare.

Report of Case

Late in September, 1959, a case of severe parathion poisoning occurred in the Phoenix, Ariz., area. The victim, a 29-year-old white man was employed by an aerial crop-dusting service as loader and flagman. On the morning of his illness, he had loaded 2% parathion dust for 4 hours, ending at 9 A.M. He was driven to the home air strip and became dizzy and nauseated on the way, vomiting on arrival. About 30 minutes later, his co-workers found him in the bunkhouse in a state of near collapse, gave him 2 atropine tablets (1/50 grain [1.2 mg.]), and took him to a physician.

His fellow employees had not felt that the patient's exposure to parathion that morning was massive; they had noted throughout the season that he habitually disregarded the recommended safety precautions. He was very careless in personal hygiene, did not take daily showers after exposure, and frequently was observed smoking and eating without washing his hands after handling parthion. He had worked as a loader and flagman from May until the time of his illness, and for an appreciable part of this time he had sustained daily exposures to parathion.

On his arrival at the physician's office, about 30 minutes later, a strong odor of parathion was noted on the patient's clothing; he had pinpoint pupils and was perspiring profusely. The patient was given 1/75 grain (0.8 mg.) atropine intra-

Cholinesterase Activities and Paranitrophenol Excretion Rates Following Intravenous Administration of 1 Gm. of 2-Pyridine Aldoxime Methiodide

Time, hr	Cholinesterase Activity * (Δ pH/Hr) RBC	Plasma	Urinary Paranitrophenol Excretion μg/Hr
	(1.0 Gm. Pyridine-2-Aldoxime-Methiodide I.V.)		
¼	0.32	0.04	Incontinent
4	0.32	0.10	Incontinent
8	0.31	0.08	Contaminated
16	0.24	0.16	
18	0.19	0.10	53
27	0.35	0.10	108
42	0.40	0.10	
44			74
54			47

* Cholinesterase activities were determined by the modified micro method of Michel.[9]

muscularly and admitted to the hospital at approximately 12 noon. Upon admission, the patient was given a warm bath and 2 mg. of atropine intramuscularly. The attending physician called the Phoenix Toxicology Unit of the Communicable Disease Center and requested advice as to treatment.

The patient's condition was growing worse; at this time, he had pinpoint pupils, excessive salivation, profuse perspiration, moist respiration, clonic convulsive movements, and incontinence. He was disoriented and unable to speak intelligibly. His blood pressure was elevated (220/110), and his pulse was rapid and irregular (140). The patient was given an additional 2 mg. of atropine intravenously without noticeable improvement.

In view of his critical condition, 1 gm. of 2-PAM in 20 ml. of saline was added to 500 ml. of 0.2% saline and infused intravenously, beginning at 2:15 P.M. The infusion was given rapidly for the first 5 minutes and later slowly, requiring a total of about 35 minutes. A dramatic improvement was noted within 10 minutes of starting the infusion. At that time it was estimated that one-third of the dose had been given. The patient became rational; convulsive movements ceased, and excessive salivation and perspiration stopped. Bladder control was still incomplete 4 hours after 2-PAM was administered. Control probably was achieved within 8 hours, although the 8-hour urine sample was contaminated by feces.

The patient was given an additional 2 mg. of atropine intravenously, and for the next 8 hours he received 1/150 grain (0.4 mg.) oral atropine hourly and thereafter 1/150 grain of atropine every 4 hours for the next 2 days. He also was given a sedative (phenobarbital) for restlessness.

The fact that severe parathion poisoning had been present was indicated by the clinical symptoms, and the massiveness of exposure was indicated by the high level of paranitrophenol in the urine (Table) as well as the finding on the third hospital day of 12 to 70μg. of parathion in 5 separate alcohol washes of the hands and body. The red cell cholinesterase activity (Table) obtained after administration of 2-PAM in this case was (*a*) higher than would be expected in comparison with the degree of illness, and (*b*) higher than would be expected in relation to the plasma cholinesterase following poisoning by parathion. Both relationships are indications that acetylcholinesterase (found in the red cells) was reactivated by 2-PAM. The relative specificity of 2-PAM as a reactivator of acetylcholinesterase as well as the clinical benefit of treatment with this drug in this case confirm earlier findings.[4,7,8]

The patient improved rapidly after administration of 2-PAM and was discharged from the hospital 5 days after exposure. At the time of discharge, he was asymptomatic and his cholinesterase values were rising. One week later, his cholinesterase values were in the lower region of the normal range (RBC 0.43, plasma 0.71Δ pH/hr.).

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Toxicology of Abate in Laboratory Animals

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AS THE NEED for a chemical compound to control the breeding of *Aedes aegypti* mosquitoes in drinking water became essential to the success of the *A aegypti* eradication program of the Public Health Service Communicable Disease Center, a series of compounds was tested for biological effectiveness against larvae of *A aegypti* at the Communicable Disease Center laboratories in Savannah, Ga. One compound, Abate (O,O,O′,O′-tetramethyl O,O′-thiodi-*p*-phenylene phosphorothioate), showed a combination of remarkable effectiveness as a larvicide when added to water at the rate of 1 ppm and low mammalian toxicity in initial studies. The formulation used to treat water actually releases approximately 0.5 ppm soon after treament, and that concentration gradually decreases with time. A series of toxicological investigations in laboratory animals was planned and executed for Abate, and the results are reported here.

Materials and Methods

All of the Abate used in these experiments (except the tests on dogs) was technical material. Three separate manufacturing batches of technical Abate designated A, B, and C were examined, but the first was studied more thoroughly, and it is the one referred to in this report unless another is specified. The material given to dogs was 43% emulsifiable spray concentrate.

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From the Toxicology Section, Technology Branch, Communicable Disease Center, Public Health Service, US Department of Health, Education, and Welfare, Atlanta. Dr. Laws is now at Johns Hopkins Hospital, Baltimore.

Reprint requests to Communicable Disease Center, Atlanta 30333 (Mr. Gaines).

Both young and adult Sherman-strain rats, adult white mice, weanling white rabbits, adult guinea pigs, adult dogs, adult white leghorn hens, chicks, and ducklings were utilized. The mammals were obtained from production facilities in the Communicable Disease Center; the birds were obtained from commercial sources. All the animals were caged individually except that some which were fed Abate in their diet were caged in groups.

For oral lethal dose (LD_{50}) studies, the technical grade compound was given by stomach tube without dilution. The same material was used for dermal toxicity studies, and the rats were confined in restraining cages so they could not lick themselves. All LD_{50} values were calculated by the method of Litchfield and Wilcoxon.[1]

To explore the possibility of potentiation in toxicity when Abate and malathion are given together, groups of two adult female rats each were given both compounds in the following proportions of the LD_{50} value of each: 1/2, 1/4, 1/8, and 1/16.

Details of dosages used in various studies of subacute toxicity are shown in Table 1. For repeated oral administration by stomach tube, technical Abate was mixed with peanut oil in varying concentrations to provide a standard dosage volume of 0.005 ml/gm of body weight. For the studies where Abate was placed in a diet to be taken ad libitum, technical Abate was mixed with a small amount of standard feed, and the concentration was adjusted by dilution. Complete details of the technique of dietary formulation are given by Gaines and Kimbrough.[2] Dosages were determined by food consumption measurements.

When Abate was administered in drinking water, the solutions were prepared fresh every 24 hours. In collecting samples from the drinking pans for chemical analysis, an effort was made to avoid water near the bottom of each pan and to take water near the surface, which a dog might lap up and drink. The analyses in-

dicated that four hours after preparation of the solution, there was 7.1 to 8.7 ppm of Abate in the 10-ppm watering pans and 45.1 to 52.0 ppm in the 50-ppm pans. After 24 hours there was 5.3 to 7.1 ppm in the 10-ppm pans and 41.0 to 51.9 ppm in the 50-ppm pans. The intake was approximately 0.6 to 0.8 mg/kg/day and 3 to 4 mg/kg/day at the two dosage levels.

In studies of the possible effects of Abate on reproduction, male and female rats were started on diets containing 500 ppm of Abate (approximately 25 mg/kg/day) at the time they were placed together for breeding. This dosage was sufficient to cause symptoms of poisoning in some of the rats. Dosage was maintained throughout mating, gestation, parturition, and lactation. The animals were kept under standard colony conditions and allowed to reproduce normally.

Autopsies were performed on representative animals from each phase of the investigations; microscopic studies were included wherever indicated.

Plasma and red blood cell (RBC) cholinesterase values were determined by automated potentiometric titration at constant pH, a modification of the method described by Jensen-Holm et al.[3] Blood for this test was taken by cardiac puncture (rats) or from the median artery of the ear (rabbits) or the cephalic vein of the leg (dogs).

Results

The results for each experiment in the series are discussed individually below. In the cases were Abate produced cholinesterase depression, the RBC enzyme was always affected more rapidly and to a greater extent than the plasma enzyme; therefore, these results are presented primarily in terms of effect on RBC cholinesterase.

Acute Toxicity to White Rats and Mice.—The results are summarized in Table 2. Both male and female rats showed definite, typical signs of organic phosphorus poisoning when given single doses by stomach tube at rates as low as 750 and 500 mg/kg, respectively, although the lowest lethal dosages were much higher. Batch A was definitely less toxic than batches B and C; however, the smallest dosage of any batch that killed a rat was 4,000 mg/kg.

The acute oral LD_{50} was determined on male mice using the same technique as for the rats. The LD_{50} was 4,700 mg/kg, and the lowest lethal dose was 3,000 mg/kg.

The highest dermal dosage of Abate that is practical to apply accurately to rats is 4,000 mg/kg. This level killed two of ten rats but

Table 1.—*Dosages of Abate Used in Subacute Studies**

Species	Sex	Route and Medium	Dosage Range (mg/kg/day)	Duration† (days)
Rat	M	ST‡—peanut oil	0, 1, 10, 100	44
	M	ST—peanut oil	0, 1, 10	28
	M	Diet—regular food		
		conc (ppm): 0	0	99
		2	0.24-0.12§‖	99
		20	2.4-1.2§‖	99
		200	24-11§‖	99
		2,000	150§	99
Rabbit	M	ST—peanut oil	0.1, 1, 10	35
	M	ST—peanut oil	100	5
Guinea pig	M	ST—peanut oil	100	5
Dog	Both	Diet—water		
		conc (ppm): 0	0	129
		10	0.6-0.8§	129
		50	3-4§	129
Chicken	F	Diet—regular food		
		conc (ppm): 0	0	108
		250	7.4§	108
		500	15.3§	108
		1,000		<44
		2,000		<44
Chick	Both	ST—peanut oil	10, 25, 50	5
Duckling	Both	ST—peanut oil	10, 25, 50	5

* The number of animals used in each test is shown in Table 3.
† Duration of dosage. At high dosage levels some animals died before the test was complete.
‡ ST indicates stomach tube; conc, concentration.
§ Approximate dosage calculated from measured food intake.
‖ Rats of the age used in this study normally eat less as they grow older, thus explaining the relatively wide range of dosage rates at the three lower dietary levels, which the rats tolerated.

Table 2.—*The Acute Toxicity of Abate in Adult Rats and Mice*

Species	Sex	Route	No.	Batch	LD_{50} (mg/kg)	95% Confidence Limits (mg/kg)	Lowest Dosage (mg/kg) Tested	Lowest Dosage (mg/kg) Lethal	Survival Time* (days) Range	Survival Time* (days) Mean
Rat	M	Oral	58	A	8,600	7,166-10,320	250	6,000	1-13	6
Rat	F	Oral	50	A	13,000	11,304-16,250	375	8,000	1-18	7
Rat	F	Oral	20	B	4,000†	...	2,000	4,000	3-11	6
Rat	F	Oral	20	C	4,000†	...	2,000	4,000	3-4	4
Mouse	M	Oral	50	A	4,700	4,123-5,358	3,000	3,000	1-7	3
Rat	M	Dermal	20	A	>4,000	...	3,000	4,000	...	...
Rat	F	Dermal	20	A	>4,000	...	3,000	>4,000	...	...

* Survival time of animals that died. Groups were observed 22 days or more.
† Approximate value.

caused no deaths among the females. All the affected animals showed the classical symptoms of illness and manner of death associated with organic phosphorus poisoning.

Subacute Oral Toxicity to White Rats.—Cholinesterase values remained normal for the rats receiving 1 mg/kg/day throughout the 44 days of the experiment.

The rats receiving 10 mg/kg/day showed 31% inhibition of RBC cholinesterase after 14 days and 47% inhibition after 44 days. These animals never showed symptoms of organic phosphorus poisoning.

The rats on 100 mg/kg/day developed typical symptoms of organic phosphorus poisoning after three doses when their RBC cholinesterase was inhibited by 64%. Gradual recovery from symptoms occurred while dosing progressed, even though the RBC cholinesterase continued to fall to 87% inhibition after 11 days of dosing. At this point some of the rats were allowed to recover for 32 days without receiving Abate and RBC cholinesterase had returned to a level of 27% inhibition at the end of this time.

The ten rats fed Abate at a dietary level of 2,000 ppm all developed signs of organic phosphorus poisoning, and all showed 100% inhibition of RBC cholinesterase after four days. Eight of the group died between the fifth through the tenth days, but the remaining two survived the 99 days of dosage in spite of continued 100% inhibition of RBC cholinesterase and 80% inhibition of plasma cholinesterase.

None of the animals receiving less than 2,000 ppm developed any symptoms of poisoning. Those on diets containing 200 ppm Abate experienced a progressive inhibition of RBC cholinesterase which was almost complete after two weeks on the diet but then improved gradually to a level of 71% inhibition at the end of the 99 days. No significant cholinesterase inhibition occurred in the groups receiving 20 or 2 ppm of Abate in their diets (mean dosages of 1.8 and 0.18 mg/kg/day, respectively). As might be expected, Abate was slightly less toxic when given in the diet than when given daily by stomach tube.

Findings of gross and microscopic pathology of rats from all the above groups were unremarkable.

Additional studies were made in rats to detect possible differences among the three batches. The results (Table 3) confirmed the relevant ones already stated for batch A but failed to reveal any significant difference among the batches.

Oral Toxicity to Rabbits.—No significant inhibition of cholinesterase occurred in the groups receiving 0.1 or 1 mg/kg/day of Abate throughout the 35 days of the experiment.

After seven days of dosage, the rabbits on 10 mg/kg/day had developed 26% inhibition of RBC cholinesterase, and inhibition increased to 47% at the end of 35 days of dosage. None of these animals developed symptoms of organic phosphorus poisoning.

Eight rabbits were placed on the dosage of 100 mg/kg/day for five days. Three of these animals died, apparently of Abate poisoning. Of the seven rabbits in this group that were autopsied, two showed focal necrosis and two showed diffuse necrosis of the liver. Liver necrosis was not observed in the autopsies of six control rabbits. Neither liver necrosis nor any other pathological evidence of poisoning was present in rabbits on dosages of 10, 1, and 0.1 mg/kg/day for 35 days. The liver injury may or may not be

Table 3.—*Effect of Different Oral Dosages of Abate on Several Species**

Species	Batch	Dosages Producing no Illness (mg/kg/day) × days treated	Batch	Dosages Producing Illness or Pathology (mg/kg/day) × days treated
Rat	A	0.12 × 99† (12/12, no inhibition of ChE†)	A	100 × 11 (7/7, severe inhibition of ChE; initial illness followed by recovery during dosage)
	A	0.12 × 99 (10/10, no characteristic pathology)	A	150 × 99 (8/10 died in 5-10 days but showed no characteristic pathology)
	A	1.2 × 99† (12/12, no inhibition of ChE)	A	150 × 99 (0/11 died, but 11/11 with symptoms and severe ChE inhibition)
	A	1.2 × 99 (10/10, no characteristic pathology)	A	500 × 4 (4/4 died)
	A	1 × 28 (8/8, no inhibition of ChE)		
	B	1 × 28 (8/8, no inhibition of ChE)		
	C	1 × 28 (8/8, no inhibition of ChE)		
	A	1 × 44 (7/7, no inhibition of ChE)		
	A	10 × 44 (7/7, moderate inhibition of ChE)		
	A	10 × 28 (8/8, moderate inhibition of ChE)		
	B	10 × 28 (8/8, moderate inhibition of ChE)		
	C	10 × 28 (8/8, moderate inhibition of ChE)		
	A	11 × 99† (14/14, marked inhibition of ChE)		
Rabbit	A	0.1 × 35 (4/4, no inhibition of ChE and no pathological changes in the liver)	A	10 × 30 (1/3, mild changes in liver pathology)
	A	1 × 35 (4/4, no inhibition of ChE and no pathological changes in the liver)	B	10 × 30 (1/4, mild changes in liver pathology)
	A	10 × 35 (4/4, moderate inhibition of ChE and no pathological changes in the liver)	C	10 × 30 (2/3, mild changes in liver pathology)
			A	100 × 5 (4/12 died; 6/12 showed focal and 2/12 showed diffuse necrosis of liver)
			B	100 × 5 (1/4 died; 1/4 showed focal and 1/4 showed diffuse necrosis of liver)
			C	100 × 5 (1/4 died; 1/4 showed diffuse necrosis of liver)
Guinea pig	A	100 × 5 (5/5, normal)		
Dog	Conc§	0.6 × 129 (2/2, normal ChE)		
	Conc§	3.0 × 129 (2/2, marked inhibition of ChE)		
Chicken	A	7.4 × 109 (2/2, normal)	A	15.3 × 109 (2/2, leg weakness after 30 days dosage)
	A	125 × 1 (10/10, normal)	B	125 × 1 (3/10, very mild, brief leg weakness)
			C	125 × 1 (3/10, very mild, brief leg weakness)
			A	250 × 1 (2/6, leg weakness)
			B	250 × 1 (1/2, leg weakness)
			C	250 × 1 (2/2, leg weakness)
			A	500 × 1 (11/11, leg weakness)
			B	500 × 1 (2/2, leg weakness)
			C	500 × 1 (2/2, leg weakness)
			A	1,000 × 1 (5/10 died; leg weakness in all survivors)
Chick Duckling	A	10 × 5 (6/6, normal)	A	25 × 5 (2/6 died)
			A	10 × 5 (4/5, mild illness; recovery during dosage)
			A	25 × 5 (5/8 died)

* The number of animals showing the condition specified is expressed as a fraction of the number tested except that in some instances not all members of a group were tested for cholinesterase activity. Each denominator represents a group; no group is listed twice.

† ChE indicates Cholinesterase activity; conc, concentration.

‡ This was the dosage received at 99 days. Food consumption and, therefore, dosage were higher at the onset of feeding when the animals were younger.

§ The material given to dogs was 43% emulsifiable spray concentrate.

accompanied by necrotic lesions in the gallbladder wall or the gastrointestinal tract.

Additional studies were carried out to detect possible differences among the three batches of Abate. When five doses were given by stomach tube at the rate of 100 mg/kg/day, the results (Table 3) were similar to those already described for batch A, and there was no significant difference in the character or degree of pathological changes produced by the different batches. Contrary to the finding in earlier tests, mild pathological changes were found in the livers of some animals that received 10 mg/kg/day, including those that received batch A.

Oral Toxicity to Guinea Pigs.—None of a group of male guinea pigs given a daily dose of 100 mg/kg of Abate by stomach tube for five days showed any sign of organic phosphorus poisoning. The guinea pigs were killed at the end of five days, and autopsy findings were unremarkable. Cholinesterase levels were not investigated.

Oral Toxicity to Dogs.—None of the dogs showed any sign of poisoning. There was no effect of Abate on the plasma cholinesterase of any of the dogs nor on the RBC cholinesterase of either of the dogs given 10 ppm Abate. The RBC cholinesterase of the male dog given 50-ppm Abate fell to 67% of normal during the first week and to 22% of normal in 129 days. The RBC cholinesterase of the female given 50 ppm Abate remained normal through the 60th day, but fell to 50% of normal by the 90th day and remained at that level through the 129th day.

Production of Leg Weakness in Chickens.—The ten hens given single subcutaneous doses of Abate at the rate of 1,000 mg/kg developed leg weakness the day of dosage, and five of the ten died. All survivors had leg weakness, which persisted from 6 to 38 days with a mean persistence of 26 days. All had recovered by 38 days after dosage. The hens receiving 500 mg/kg all developed leg weakness the day of dosage, and all survived. Leg weakness persisted from 11 to 31 days with a mean persistence of 15 days. All these hens had recovered by 31 days after dosage. The response of the chickens to Abate at these dosages were similar to that seen for malathion[4] in that onset of leg weakness was immediate and recovery occurred with time.

All hens given Abate at the dietary concentration of 2,000 and 1,000 ppm died in 30 to 43 days. The hens fed 500 ppm (mean dosage of 15.3 mg/kg/day) developed leg weakness after 30 days of dosage. The hens given 250 ppm (mean dosage of 7.4 mg/kg/day) did not show symptoms of leg weakness at any time during the 108 days of dosage.

Additional acute tests were done to detect possible differences among the three batches of Abate. The results (Table 3) indicate no significant difference among the batches. The lowest dosage tested (125 mg/kg) produced a very mild effect that disappeared within only six days.

Oral Toxicity to Chicks and Ducklings.—All the chicks and ducklings receiving Abate by stomach tube at the rate of 50 mg/kg/day died. Two of six chicks and five of eight ducklings on 25 mg/kg/day died, while the remainder on this dosage all showed signs of organic phosphorus poisoning. The sick birds were generally weak, but typical leg weakness was not observed.

The chicks on 10 mg/kg/day showed no ill effects of any kind, while the ducklings on the same dosage developed minimal signs of poisoning over the first two days but recovered and appeared normal by the end of the five days of dosage.

Autopsy findings for chicks and ducklings were unremarkable. Cholinesterase levels were not investigated in the chicks and ducklings.

Acute Oral Toxicity to Rats of Combinations of Abate and Malathion.—All of the rats given dosage levels of 1/8 LD_{50} Abate plus 1/8 LD_{50} malathion or greater died. Those receiving 1/16 LD_{50} Abate plus 1/16 LD_{50} malathion showed no clinical effect. The results indicate approximately a fourfold potentiation of toxicity where the two compounds are given together at levels approaching the LD_{50} values.

Effect on Reproduction in Rats.—There was no significant difference in number of litters produced (15 from 15 matings), litter size (average, 10.5), viability of young, or incidence of congenital defects between rats receiving Abate at a dietary level of 500

ppm and normal members of the rat colony, even though the dosage caused signs of poisoning in some rats.

The RBC cholinesterase of the mothers was inhibited by 90% after 48 days of dosage, while that of their healthy 21-day-old young was inhibited by 30%.

Summary

In laboratory animals Abate produces signs, symptoms, and type of death typical of those associated with other organic phosphorus compounds. In large enough doses Abate will inhibit cholinesterase, and it does this more promptly and to a greater extent for RBC cholinesterase than for plasma cholinesterase.

The acute oral lethal dose (LD_{50}) for Abate in rats and male mice is 4,000 mg/kg or greater. Restrained female rats tolerate a dermal dose of Abate of 4,000 mg/kg without clinical effect, but the same dosage killed two of ten males.

Rats, rabbits, guinea pigs, and chickens tolerate a daily oral dosage of Abate of 10 mg/kg without observable clinical effect, and dogs tolerate a daily dosage of 3 to 4 mg/kg, the highest rate at which they were dosed. Rats and rabbits tolerate at a dosage of 1 mg/kg/day for extended periods of time without detectable effect on cholinesterase.

Focal or diffuse necrosis of the liver was present in some rabbits given Abate at the rate of 100 mg/kg/day for five days, and mild pathological changes occurred in some that received 10 mg/kg/day for 30 days. No liver necrosis or other characteristic pathology of the liver was present in rabbits given 1 and 0.1 mg/kg/day for 35 days, and no relevant pathological effects of any kind were found in the other species studied.

Abate in a dosage of 500 mg/kg or higher produced a rapid onset of leg weakness in chickens from which the survivors recovered within 36 days or less. A single dose at the rate of 125 mg/kg produced a very mild leg weakness in a few chickens, but they recovered promptly. When Abate was fed to hens daily, those receiving an average dosage of 15.3 mg/kg/day developed leg weakness after 30 days, but hens that received an average dosage of 7.4 mg/kg/day did not show symptoms of leg weakness at any time, although they were fed Abate for 108 days. Thus, the effect of Abate on chickens is similar to that of malathion.

Abate and malathion show an approximately fourfold potentiation of toxicity when the two are given together at levels approaching the LD_{50} values.

Male and female rats maintained on a dietary intake of Abate large enough to produce marked inhibition of cholinesterase and some symptoms of poisoning reproduced normally with no decrease of number of litters, litter size, or viability of young and with no increase of congenital defects.

Batches of technical Abate differ somewhat in their acute oral LD_{50} values, but the toxicity of all tested was low. More important, there were no significant differences in the effects of different batches in regard to their ability to produce pathological changes in the liver in rabbits, leg weakness in chickens, or cholinesterase inhibition in rats following repeated dosage.

Richard Moore and Ralph Linder gave technical assistance with the animals; Miss Florence Whitfield performed the cholinesterase determinations, and Mrs. Martha Bey assisted in the preparation of tissue for microscopic study.

The technical Abate and the emulsifiable concentrate of the compound used in this study was supplied without charge by the American Cyanamid Laboratories, Princeton, NJ.

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Biochemical Effects in the Rabbit of Repeated Administration of a Mixture of DDT, Carbaryl, and Parathion

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Some biochemical effects of the repeated administration to rabbits of DDT (5 mg/kg), carbaryl (5 mg/kg), and parathion (0.5 mg/kg) were studied during 222 days. No significant changes were found in the blood or urine levels of amino acids. The tubular phosphorus reabsorption remained unaltered. The blood coagulation time was considerably shortened in the treated animals. The urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA) and 4-hydroxy-3-methoxymandelic acid (VMA) was higher in treated animals as compared to that of controls, indicating an increased rate of metabolism of serotonin and catecholamines, respectively. This increased metabolism is in turn believed to be a consequence of increased biosynthesis and release of biogenic amines caused by a "stress mechanism" induced by the administered pesticides. The amounts of 5-HIAA and VMA returned to normal 45 days after cessation of treatment.

Extensive studies and reviews have been published of the clinical aspects and symptomatology of pesticide poisoning, of the manner in which the body handles pesticides, and of residues of pesticides and their ultramicrochemical determination. In contrast, there are very few published reports of controlled experimental studies of the biochemical effects after prolonged exposure to pesticides. If any general public health problem exists because of pollution of food, water, or air with pesticides, it is at low levels of prolonged exposure. For this reason, it became important to orient research to biochemical and pharmacological effects of such exposure to pesticides.

TOCCI [1] reported that humans with occupational exposure to a wide range of unidentified pesticides develop renal tubular dysfunction, aminoacidemia, and aminoaciduria. WILLS et al. [2] administered carbaryl to human volunteers and presented data indicating that the ability of the proximal convoluted tubule to reabsorb amino acids was decreased. Our studies are an attempt to reproduce these findings

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in a laboratory animal so as to facilitate more fundamental studies of the mechanisms of action under more rigorously controlled experimental conditions than those usually possible in man. Other parameters were included as the study progressed.

Experimental

New Zealand white male rabbits weighing 1700 – 2300 g each were used throughout this investigation and were maintained on Purina rabbit chow. Five animals received a daily dose of pesticides consisting of a mixture of 5 mg of DDT*/kg body weight, 5 mg of carbaryl**/kg, and 0.5 mg of parathion***/kg, administered orally in 2 ml of peanut oil. Another group of five rabbits was treated daily with the same mixture at twice the concentration; a third group of five animals received only peanut oil and served as controls. Treatment was continued for 222 days. The amount of pesticide administered was adjusted every six weeks to correspond to the increase in weight of the animals.

The animals were kept in metabolism cages in order to obtain urine samples free from feces. Blood samples were taken by heart puncture or from the ear vein.

The α-amino acid nitrogen content of plasma was determined weekly for 14 weeks according to the method of FRAME et al.[3], with β-naphthoquinone-4-sulfonic acid as the reagent. The same procedure was used for the determination in urine after the interfering ammonia had been removed by adjusting the pH to 12.2 – 12.5 with 0.1 N sodium hydroxide and passing a stream of nitrogen into the solution for 15 – 20 min.

The tubular phosphorus reabsorption (TPR), involving phosphorus and creatinine concentrations in both plasma and urine, was used as an index of kidney function. Inorganic phosphorus in blood and urine was determined by the method of FISKE and SUBBAROW[4] and creatinine was determined by the Jaffé reaction[5].

The total plasma proteins were determined colorimetrically with biuret reagent[6] on the 13th, 16th, and 19th weeks after the beginning of treatment. The electrophoretic pattern of plasma proteins was also studied each week during the first 19 weeks; two plasma samples from each group were analyzed using acrylamide gel and Tris-HCl buffer, pH 8.1.

Hemoglobin was assayed over 100 days of treatment; whole blood catalase activity was also assayed by the method of MILLER[7] during the same period. Blood coagulation time at 14 and 21 weeks was determined by the capillary procedure; blood was allowed to flow from the ear vein into a capillary tube and coagulation was checked every 15 sec by breaking a portion of the tube.

Possible effects on carbohydrate metabolism were investigated at 19 and 21 weeks by determining the fasting serum glucose level, using the *o*-toluidine reagent[8]. The metabolism of catecholamines was investigated after 100 days of treatment by determining the metabolite of epinephrine and norepinephrine, 4-hydroxy-3-methoxymandelic acid (VMA), in the urine according to the procedure of PISANO et al.[9]. The major metabolite of serotonin (5-hydroxytryptamine), 5-hydroxy-3-indoleacetic acid (5-HIAA), was determined in the urine by the method of UDENFRIEND et al.[10] at the same time intervals described for VMA. In addition, both VMA and 5-HIAA were assayed 45 days after the cessation of treatment. Blood serotonin was also determined by the method of UDENFRIEND et al.[11].

The data in this paper were analyzed by one-way analysis of variance to test for differences in treatment means. The *F*-statistic was used to find the significance level. When the differences were significant, the appropriate *P* value was determined.

Results

Throughout the 222 days of treatment, no clinical signs characteristic of pesticide poisoning were observed. The treated animals gained weight at a rate similar to that of the control animals.

After 14 weeks of treatment the levels of amino acid nitrogen in both plasma and urine were similar to those at 0 time and no significant difference was observed between the three groups (Table 1). The differences in the amino acid nitrogen:creatinine ratios were also insignificant. Similarly, TPR values at 14 weeks did not vary significantly from those at

* DDT (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane), technical grade, was obtained from Allied Chemical Corporation, New York, New York.

** Carbaryl (1-naphthyl methylcarbamate), technical grade, 99.9%, was obtained from Union Carbide Corporation, New York, New York.

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Group	Weeks of treatment	Amino acid nitrogen		Amino acid nitrogen: creatinine	TPR
		Plasma [mg/100 ml]	Urinary [mg/24 hr]		
Control	0	6.2	6.4	0.053	0.90
	14	6.0	7.5	0.069	0.89
Low-dose	0	6.0	7.5	0.065	0.91
	14	5.7	5.9	0.055	0.89
High-dose	0	5.9	7.1	0.063	0.91
	14	5.9	6.1	0.062	0.93

Table 1. Amino acid nitrogen and tubular phosphorus reabsorption of pesticide-treated rabbits (all data are means of 4–5 animals).

0 time and values for the treated groups did not differ from those of the control group, indicating that the reabsorption of phosphorus by the tubules was not affected by the pesticide treatment (Table 1). The amounts of creatinine excreted into urine per 24 hr were fairly consistent; the ranges were 92–137, 84–128, and 81–130 mg for the control, low-dose, and high-dose animals, respectively.

After 13, 16, and 19 weeks of treatment, the ranges of the total plasma protein concentrations (g/100 ml plasma) were 7.24–8.36, 7.11–7.98, and 7.30–8.36 for the control, low-dose, and high-dose animals, respectively. These values were not significantly different for any group. No detectable change in the electrophoretic patterns of the plasma proteins could be observed at any week during the first 19 weeks.

Erythrocytes from all animals (control and treated) had the same degree of fragilty. Hemoglobin concentration was fairly constant over a period of 100 days. During the same period, the activity of the closely related hemoprotein catalase remained unaltered, indicating that the biosynthesis of these hemoproteins in the bone marrow was not impaired.

During the 15th to 17th weeks of treatment, fasting serum glucose values were 64–76, 66–68, and 64–79 mg/100 ml for the control, low-dose, and high-dose animals, respectively, showing that the administered pesticides did not significantly affect carbohydrate metabolism. Nitroprusside reagent did not give a positive reaction for acetone bodies in the urine of any animal.

Table 2 shows that blood coagulation time was reduced in the treated animals after 14 and 21 weeks. These differences were highly significant ($P<0.01$ for both treated groups as compared with the control group). However, the effect on coagulation time was not progressive during the time of study.

Weeks on treatment	Control	Low-dose	High-dose
14	4.3±0.1	2.5±0.1	2.4±0.1
21	3.9±0.3	2.9±0.1	2.9±0.1

Table 2. Blood coagulation time in minutes (all data are means ± SE of 4–5 animals).

After 100 days of treatment with the pesticide mixture the excretion of VMA by the treated animals was 190–300% higher than that of the controls. Levels of this metabolite remained fairly constant and elevated during the period of observation, as is evident by the values at 200 days. Administration of the pesticides was stopped after 222 days; after 45 days of recovery the VMA had returned to or approached pre-treatment levels. Some treated animals (one-third of all treated) did not excrete high amounts of urinary VMA, resulting in the relatively high values for the standard errors. These results are shown in Table 3.

Group	[μg V MA/mg creatinine]		
	Days on treatment		Days of recovery
	100–150 [a]	200 [b]	45 [b]
Control	5.4±0.2	4.9±0.6	6.3±0.7
Low-dose	11.4±1.4	11.5±1.3	8.2±2.6
High-dose	16.2±1.4	15.3±1.4	5.4±0.8

Table 3. Urinary excretion of 4-hydroxy-3-methoxymandelic acid (VMA). [a] Group means ± SE of 7 consecutive weekly determinations. [b] Means ± SE of 3–5 animals.

Group	μg 5-HIAA/mg creatinine		
	Days on treatment		Days of recovery
	100–150 [a]	200 [b]	45 [b]
Control	4.9±0.1	7.2±0.5	5.4±0.1
Low-dose	12.8±0.6	15.0±8.1	7.9±1.4
High-dose	13.8±0.7	23.6±5.2	4.7±0.9

Table 4. Urinary excretion of 5-hydroxy-3-indoleacetic acid (5-HIAA). [a] Group means ± SE of 7 consecutive weekly determinations. [b] Means ± SE of 3–5 animals.

Table 4 shows that the treated animals also excreted significantly higher amounts of 5-HIAA than

did the control group. As with VMA, the 5-HIAA values returned to or approached normal 45 days after cessation of treatment. Blood serotonin values of the pesticide-treated groups were not significantly different from those of the untreated group.

Discussion

Prolonged administration of a mixture of parathion, carbaryl, and DDT to rabbits did not alter the amino acid level in blood or urine. It is possible that species differences may account for the increased level of amino acids in the blood and urine which has been reported in humans exposed to various unidentified pesticides [1]. These humans, who were occupationally exposed to the pesticides also had kidney insufficiency, as indicated by low phosphorus reabsorption [1]. The TPR values we obtained for treated animals were all within the normal range and there was no indication of such insufficiency. The protein and carbohydrate metabolism apparently remained unaffected.

The reduction in coagulation time observed in the treated animals is believed to be one of the manifestations of non-specific stress. Stress provokes a significant response of the pituitary-adrenal axis with the resultant increase in the release of adrenocortical hormones. These hormones are known to distinctly shorten the coagulation time.

The increase in urinary excretion of VMA is probably related to an increased rate of metabolism of catecholamines. Stress, which is presumably caused by the repeated administration of the pesticides, induces an increased release of catecholamines. This might result in an increased rate of metabolism with subsequent higher levels of VMA in the urine, and finally a depletion of the amine stores of certain tissues and/or acceleration of the amine biosynthesis processes. STAVINOHA and RIEGER [12] reported that an acute dose of DDT increased the urinary excretion of epinephrine and norepinephrine in rats. The adrenals were believed to contribute to this increase, since they were found to be depleted of the amines. An indirect effect through the central nervous system on the adrenals has not been precluded.

The elevation of urinary 5-HIAA in the pesticide-treated animals is probably the result of an increased rate of serotonin metabolism. Various stressors are known to elevate the brain level of biogenic amines. It is suggested that the repeated administration of pesticides may be associated with "stress", producing an elevation of brain serotonin and consequent increase in its biosynthesis and degradation [13]. 5-HIAA is transferred directly from the brain to the plasma [14]. In support of the "stress hypothesis" is the fact that the increase in urinary 5-HIAA was no longer detectable after the withdrawal of the stressor (cessation of treatment). The animals which excreted the highest amounts of VMA also eliminated the highest amounts of 5-HIAA, suggesting that a common mechanism underlies the alteration in the metabolism of these biogenic amines.

Fig. 1 presents some possible mechanisms which may account for alterations of the metabolism of biogenic amines. The pesticide(s) may act directly on the central nervous system as stressor, resulting in increased metabolism of the amines. Through the

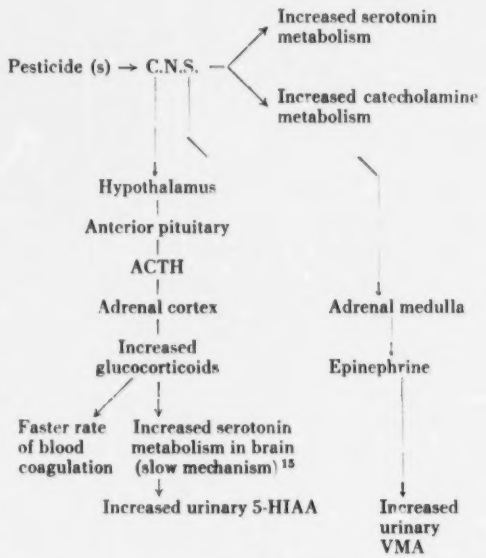

Fig. 1. Possible mechanisms for increased urinary excretion of metabolites of biogenic amines.

[12] W. B. STAVINOHA and J. A. RIEGER, JR., Toxicol. appl. Pharmacol. 8, 365 [1966].
[13] A. S. WELCH and B. L. WELCH, Biochem. Pharmacol. 17, 699 [1968].
[14] N. H. NEFF, T. N. TOZER, and B. B. BRODIE, J. Pharmacol. exptl. Therap. 158, 214 [1967].
[15] G. CURZON and A. R. GREEN, Life Sci. 7, 657 [1968].

central nervous system, increased release of epinephrine from the adrenal medulla might result in increased urinary VMA. Increased glucocorticoid secretion might be the result of an indirect action on the adrenal cortex through the central nervous system.

Most of the treated animals showed varying degrees of diuresis (the increase in urine output exceeded 100% in some animals). The mechanism by which this diuretic response is induced is not known. The findings reported in this paper might be the result of interaction of one or more of the administered pesticides. However, there is some evidence that DDT largely contributes to these effects (unpublished data). Further studies are necessary to clarify the various mechanisms.

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PB 279 278

Pharmacological Approach to Toxicology

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The technical literature on pesticides indicates there is extensive work being conducted and published on methods of analysis, extent of human exposure, symptomatology of poisoning and levels of pesticide residues in biological tissues and residues in all phases of our environment. A limited review of the literature establishes the need for greater emphasis on more properly controlled studies on the biological significance of pesticide residues. These studies should keep pace with the extensive development of chemical methodology and its applications.

Since the general public is exposed to low levels of pesticides, biological studies must emphasize the possible effects of prolonged or repeated low-level exposure to pesticides on the functional aspects of sensitive physiological systems. Application of pharmacological methodology to these functional systems would facilitate a more sensitive evaluation of possible toxic effects.

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National Communicable
Disease Center
Atlanta, Ga. 30333

TOXICOLOGY AND APPLIED PHARMACOLOGY, 12(2):285, March, 1968.

Chlorinated Hydrocarbon Insecticides in Plasma and Milk of Pregnant and Lactating Women. A. CURLEY and R. KIMBROUGH, Toxicology Laboratory, Pesticides Program, National Communicable Disease Center, U.S. Public Health Service, Atlanta, Georgia. (Sponsor: W. J. Hayes, Jr.)

Five pregnant women were studied for the estimation of chlorinated hydrocarbon insecticide content in their blood and milk. Blood samples were obtained between days 30 to 150 and 240 to 263 of pregnancy, and between days 1 to 6, 35 to 55, and 90 to 115 postpartum. Milk samples were collected 3–6 days postpartum. Other milk samples were collected simultaneously with the other two postpartum blood samples. Extracts from these samples were analyzed by electron-capture gas-liquid chromatography.

Nine chlorinated insecticides were detected in plasma and milk. The concentrations in plasma were within the lower part of the range previously reported for healthy, nonpregnant women of the same socioeconomic group and city, who had no known special exposure to pesticides. However, the mean concentrations (ppm) of seven compounds—*p,p'*-DDT (0.0028), *o,p'*-DDT (0.0008), *p,p'*-DDE (0.0049), *o,p'*-DDE (0.0003), *p,p'*-DDD (0.0003) and total beta and gamma-BHC (0.0018)—in the plasma were significantly lower than those of the nonpregnant women. This finding suggests but does not prove that pregnancy leads to lower plasma levels of insecticides. The dieldrin concentration (0.0012 ppm) was not significantly different from that in nonpregnant women. Heptachlor epoxide was detected also, but no data are available for this compound in nonpregnant women. A pronounced decrease in the plasma concentration of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDD, total beta and gamma-BHC and dieldrin was observed 1–6 days postpartum in all the women. Subsequently, the concentration of these compounds increased to approximately the levels found during pregnancy. The concentration of *o,p'*-DDT did not show this trend. Although there was much individual variation, the mean excretion levels of *p,p'*-DDT, *p,p'*-DDE, *o,p'*-DDE, dieldrin, and total beta and gamma-BHC in the milk increased. Little correlation was found between the concentrations of total-DDT, total-BHC, heptachlor epoxide, and dieldrin in the plasma and milk.

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PB 279 280

THE TOXICITY OF POLYCHLORINATED POLYCYCLIC COMPOUNDS AND RELATED CHEMICALS

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667
Environmental Protection Agency

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INTRODUCTION

Only the small group of the polychlorinated polycyclic compounds given in Figures 1 and 2 will be reviewed in this article. Several years ago when this laboratory was the Atlanta Toxicology Branch of the Food and Drug Administration, concern was voiced over the possible long-term effects of polychlorinated biphenyls, and we began to study the toxicity of Aroclor 1254 and Aroclor 1260. About this time a follow-up study was performed on a group of workers in New Jersey who were engaged in the production of 2,4,5-T.[1] These workers had very severe chloracne and had previously been shown to have porphyria.[2] During that same time, a petition was filed with the Food and Drug Administration for a residue level of hexachlorophene on certain food crops. Hexachlorophene, as well as 2,4,5-T, is made from trichlorophenol, although the chemical reactions are quite different. When we checked the available literature on the toxicity of hexachlorophene we felt that adequate toxicity data by present standards were not available, particularly not if the compound was meant to be ingested. Furthermore, Larson[3] had pointed out that hexachlorophene was more toxic than commonly acknowledged.

Hexachlorophene and similar chemicals behave differently from such polycyclic polychlorinated compounds as the chlorinated biphenyls (PCBs). Hexachlorophene in particular is acutely much more toxic than the PCBs and is fairly rapidly eliminated from the body, while the PCBs have a very low acute toxicity, but are very poorly metabolized, particularly those with more than four chlorine atoms on the phenyl rings. The long-term effects are therefore more critical for compounds such as PCBs. The chlorinated dibenzodioxins and furans, some of which are extremely toxic on an acute basis, may also show persistence and their long-term effects may be equally as important. These observations show that it would be erroneous to make predictions on long-term effects from results obtained in acute toxicity studies.

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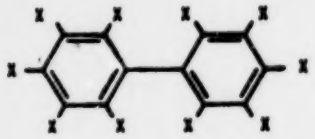
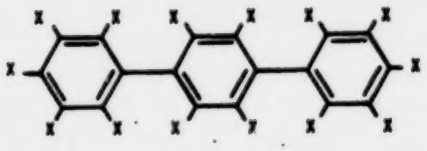
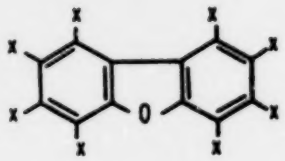


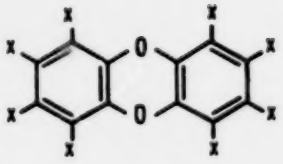
Polychlorinated Biphenyls (PCB's)



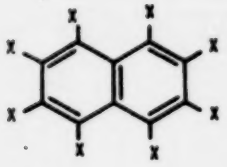
Polychlorinated Terphenyls



Chlorinated Dibenzofurans

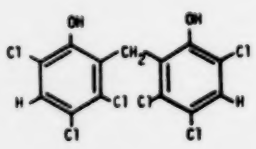


Chlorinated Dibenzodioxin

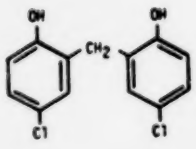


Polychlorinated Naphthalenes

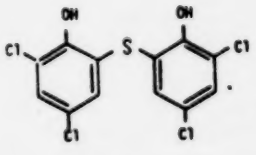
FIGURE 1. The materials shown in this figure are mixtures of compounds in which several but usually not all of the positions (-X) are chlorine and the rest are hydrogen. The properties of the materials depend on the degree of chlorination of the mixture. All are insoluble in water but soluble in varying degrees in organic solvents and in fats. The trade name for the U.S. product (Monsanto) of the chlorinated biphenyls and terphenyls is Aroclor. The trade name is followed by the number. For the biphenyls, the first 2 digits of this number are 12, and for the terphenyls, 54. In each instance the last 2 digits give the percentage of chlorine.



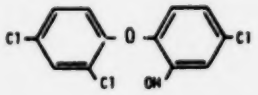
HEXACHLOROPHENE
2,2'-Methylene-bis(3,4,6-trichlorophenol)



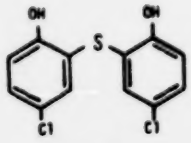
DICHLOROPHENE
2,2'-Methylene-bis(4-chlorophenol)



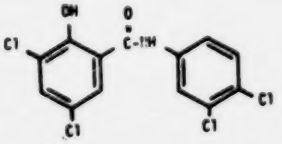
BITHIONOL
2,2'-Thio-bis(4,6-dichlorophenol)



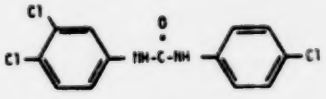
2,4,4' trichloro- 2'hydroxy-diphenyl ether
(Triclosan • Irgasan DP300)



FENTICLOR
2,2'-Thio-bis(4-chlorophenol)



3,3',4,5'-Tetrachlorosalicylanilide (TCC)



3,4,4'-Trichlorcarbanilide (TCB)

FIGURE 2. The compounds in this figure are white crystalline solids with clearly defined melting points above 160°C. They are practically insoluble in water and soluble in varying amounts in organic solvents and in fats. Those containing an -OH group, all the above excepting 3,4,4'-trichlorocarbanilide, are soluble in aqueous alkali.

The present review is an attempt to correlate various toxic effects produced by the chemicals listed in Figures 1 and 2 in order to better understand and appreciate their behavior.

The subjects covered in this article have lately enjoyed great interest and a rapidly growing number of scientific reports are available. Since a number of the chemicals discussed produce similar or related effects, their toxicology is discussed together rather than listing each compound separately, which would have led to a great deal of repetition.

A detailed discussion of the various chemical reactions, analytical methods for the determination of the compounds, is not given. The review has been written with the toxic effects of the various compounds as the focal point and the literature cited serves to illustrate these points. No attempt has been made to present a complete review of the literature in this area.

DEVELOPMENT, USAGE, AND OCCURRENCE

A description of the synthesis of *chlorinated biphenyls* (PCBs) can be found in *Liebig's Annalen* as early as 1881.[4] Successful production of diphenyl in commercial quantities was not reported until 1930.[5] At that time the chlorination of technical biphenyl was also described.[6] Biphenyl itself is fungistatic and used as a preservative for citrus fruits.[7] Chlorinated biphenyls are very stable, and have been used for protective coatings, as plastic resins or gums for varnishes, and for waterproofing and flameproofing of wood.

The excellent dielectric properties of these compounds were noted as early as 1930. Until the advent of the PCBs, mineral oil was used as a cooling and dielectric liquid impregnant for high voltage electric apparatus such as transformers and capacitors. Since mineral oil is flammable and flammable gases could also be produced, restrictions for the use and installations of these transformers had to be set, particularly for indoor installation. When mineral oils were replaced by the Aroclors, the fire and explosion hazards were eliminated. This was recognized by the National Board of Fire Underwriters and the National Electric Code was modified.[8] Use of Aroclors also greatly reduced the physical size of the capacitors.[9]

A recent summary of the varied uses of PCBs has been provided by Broadhurst.[10] With a peak production of 42,527 short tons of PCBs in 1970, Monsanto is the sole producer of Aroclors (U.S. tradename for PCBs) in the U.S. Yearly PCB production figures for the period 1960-1971 were reported by Monsanto.[11] The production increased steadily until 1970 and dropped to 20,236 short tons in 1971 due to a voluntary restriction of the use of PCBs by the company. Trade names in other countries include Kanneclor and Santhaterm in Japan, Clophen, Flenclor, Phenoclor, and Sovol in Europe.

The polychlorinated capacitor liquids, commonly called askarels, are mixtures of chlorinated biphenyls and chlorinated benzenes. A variety of other industrial applications[10] have been found for PCBs. They have served as fluids for hydraulic, gas turbine, and vacuum pumps, as heat transfer fluids, as plasticizers, adhesives, textile coatings, surface coatings in paints and varnishes, as sealants, and as part of formulations to prepare pressure sensitive records and color copying papers. The extent of the current use of PCBs in printing processes is unknown. Other suggested uses over the years have included catalyst carrier for polymerization of olefins, conversion of water permeable soil to a nonpermeable state, and combined insecticide and bactericide formulations. Mixtures of chlorinated biphenyls and chlorinated naphthalenes have been employed to insulate electric wires and cables, particularly when they were used in the mining industry and on warships.[12]

Residues of polychlorinated biphenyls have been reported in the environment by a number of authors in different parts of the world. These reports were usually from areas where industrial pollution was responsible for the environmental contamination. Holden,[13] after finding PCBs in marine fish and mollusks from Scottish waters, noticed that they were generally highest in samples from the Firth of Clyde and he discovered that the PCB contamination probably originated from industrial sewage. The sewage sludge was transported from two Glasgow purification works by boats and dumped in deep water six miles south of Garroch Head. The disposal of sewage sludge on land could lead to the introduction of PCB residues into the terrestrial ecosystem by transfer through food chains. So far PCB residues have not been detected in fresh water fish in Scotland.

Polychlorinated biphenyls have also been found

in wildlife samples collected along the Dutch coast and the Rhine River[14] and in fish and seal in different parts of the Baltic Sea along the Swedish coast.[15]

Risebrough et al.[16] compared the presence of PCBs with that of DDT and its metabolites in various birds. These authors found very low concentrations of PCBs in wildlife species in Baja, California, Mexico, and the Gulf of California, which are very remote areas with a high ratio of DDT to PCB of approximately 9 or 10. In sea birds from the Pacific, the ratio was between 5 and 10 and at that time, PCBs were not found in eggs of the Adelie penguin from Cape Crozier, Antarctica. Relatively high PCB concentrations were found in San Francisco Bay, Puget Sound, and San Diego Bay and the ratio of DDT to PCBs in wildlife samples from this area was between 1 and 2. This is again emphasized by the findings of Dustman et al.[17] Twelve Alaskan bald eagle eggs had median PCB residues of 1.65 ppm while 11 eggs collected in Maine, Michigan, Minnesota, and Florida showed a median concentration of 9.7 ppm.

Zitko[18] found low concentrations of PCBs in different types of fish taken from several lakes and the St. John River System, New Brunswick, Canada, and the Nova Scotia banks. Fish from the Milwaukee River and Lake Michigan in the mid-west part of the U.S., where the river enters into the lake, contained varying amounts of PCBs[19] while fish taken from various small lakes in Wisconsin without industrial discharge failed to show PCBs. Large predators from Lake Michigan contained as much as 10 to 25 ppm PCBs on a fresh weight basis expressed as Aroclor 1254 and salmon eggs contained as much as 12 to 17 ppm PCBs on a fresh weight basis. Further studies by Veith[20] of the concentration of PCBs in different rivers emptying into Green Bay indicated a trend towards a decrease in PCB concentrations since the *voluntary* partial ban on PCB sales by Monsanto was implemented in September of 1970.

If PCB discharges are very high a fishkill may result such as the one in Escambia Bay, Florida in 1969.[21] In that particular episode the PCBs had entered a plant's effluent through accidental leakage of heat exchange fluid. The PCB found in this case resembled Aroclor 1254 most closely. The peak amount found in water of the Escambia River at the outfall amounted to 275 ppb and the peak concentration found in oysters from Escambia Bay was 3.0 ppm. Aroclor residues in sediment samples taken near the outfall reached 486 ppm. When the leakage from the plant was corrected, Aroclor levels gradually decreased in this area.

The wildlife portion of the U.S. National Monitoring Program analyzed starlings, mallard and black ducks, bald eagles, and fish of several species for PCBs. According to Stickel,[22] monitoring for terphenyls is also done but not for any of the other chlorinated compounds in this report. Research surveys that include fisheating birds, insectivorous birds, ospreys, woodcocks, and certain ducks also monitor only for PCBs and for pesticides. Oysters (*Crassostrea virginica*) are a good indicator of PCB and other chlorinated hydrocarbon concentrations in water and a national monitoring program in these mollusks in 15 coastal areas has been developed.[23] So far, mollusks have only very occasionally contained low levels of PCBs.

In summary, the PCB concentrations in water are low or nonexistent in remote lakes and fresh water streams and can be as high as 50 μg/l (5 ppb) or higher in polluted rivers such as the Hudson River.[24] The PCB concentration is usually higher in sediment from these polluted waters because PCBs are only very slightly soluble in water.[25] Since PCBs are stored in living matter, they are concentrated in the food chain and the usual "biomagnification" is encountered as we know it from other poorly metabolized and excreted substances. The concentration of PCBs in fish, particularly those in the upper end of the food chain from polluted rivers and lakes, is usually estimated in the ppm range and fisheating birds will have somewhat higher levels of PCBs than the fish from the same geographic location.[26] Young animals may contain less PCBs than their older counterparts of the same species in a particular location.

PCBs are also infrequently encountered in dairy products, meat, fish, and poultry in ready-to-eat human food.[27,28] Occasionally, human food and domestic animal feed have been more extensively contaminated with PCBs. In these instances, the contamination could usually be traced to local misuse of transformer fluid, leakage of PCBs from heat exchange fluid, or contamination of recycled paper with PCBs where the paper had been used for food packaging such as cereal boxes.[29] The U.S. Food and Drug Administration has recently set temporary tolerances for PCBs in certain food products (see Table 1).

Temporary Tolerances Expressed as Parts per Million (ppm) Established by U.S. Food and Drug Administration

(1) Milk (fat basis)	2.5
(2) Dairy products (fat basis)	2.5
(3) Poultry (fat basis)	5.0
(4) Eggs	0.5
(5) Complete and finished animal feeds	0.2
(6) Animal feed components	2.0
(7) Fish and shellfish (edible portion)	5.0
(8) Infant and junior food	0.2
(9) Paper food-packaging material	10.0

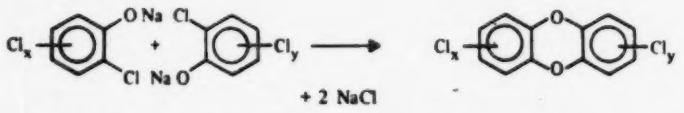
Note: The tolerance for paper food-packaging material shall not apply to such material separated from the packaged food by a functional barrier impermeable to PCB migration.
The Food and Drug Administration will provide upon request the analytical methods it will use for enforcing the temporary tolerances.

All of these aspects of PCBs in the environment have been the subject of a number of reports, reviews,[30] and conferences.[31-35]

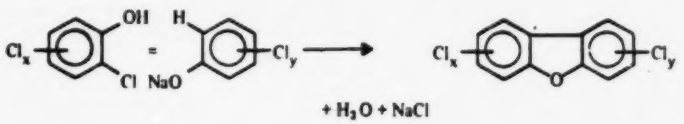
Terphenyls and chlorinated terphenyls are used as reactor coolants. The terphenyls consist of mixtures of ortho-, meta-, and paraterphenyls. Chlorinated terphenyls are produced by Monsanto and termed Aroclor, followed by numbers. The first 2 digits of these numbers are 54 and the last 2 indicate the percent of chlorine such as Aroclor 5460. Aroclor series 25 and 44 consist of mixtures of polychlorinated biphenyls and polychlorinated terphenyls.[36] According to Zitko et al.,[37] polychlorinated terphenyls have been found in environmental samples. Polychlorinated terphenyls do not elute from GLC columns under conditions used for PCBs,[38] which may explain why they have only been detected occasionally, but the way they are used probably also precludes their extensive spread in the environment.

Chlorinated dibenzodioxins and *chlorinated dibenzofurans* may occur as contaminants of cyclic chlorinated hydrocarbons.

In 1959 Tomita et al.[39] reported on the synthesis of polyhalodibenzo-p-dioxins. According to these authors, chlorophenols, when heated to about 200°C or more under alkaline conditions and pressure, will form chlorodibenzodioxins, as shown below.



Under similar conditions a chlorodibenzofuran may also be formed.



Chlorinated dibenzodioxins and chlorinated dibenzofurans are formed, or can be formed, as contaminants in the manufacture of a variety of products which use chlorophenols and chlorobenzenes as starting materials, such as 2,4,5-T, pentachlorophenol, nitrofen, hexa- and pentachlorobenzene, and tri- and tetrachlorophenol. Polychlorinated biphenyls may also be contaminated with chlorinated dibenzofurans,[40,41] and chlorinated dibenzofurans may result from polychlorinated biphenyls under UV irradiation.[42] The toxicity of these products varies depending on the position and number of chlorines attached to the phenyl rings. Products from different manufacturers may show a great deal of difference in the type and amount of contamination and different lots of the same product may vary in composition, making toxicological evaluations of these products very difficult. Rigorous production control and cleanup procedures need to be implemented so that the composition of different lots manufactured by the same company becomes more uniform.

Villanueva et al.[43] and Jensen and Renberg[44] reported the presence of chlorinated dibenzo-p-dioxin in pentachlorophenol. Hydroxynonachlorodiphenyl ether was also found and referred to as "predioxin" under the assumption that it represented a precursor of dioxin.

According to Johnson et al.,[45] the predominant dioxin in a commercial pentachlorophenol was octachlorodibenzodioxin while trace amounts of hexachlorodibenzodioxin and no tetrachlorodibenzodioxin were found. Since the technical pentachlorophenol was in some respects more toxic in animal studies, than the pure product, the production process of pentachlorophenol was changed to minimize the dioxin concentration and a new product has now been registered under the Federal Insecticide Fungicide and Rodenticide Act with a lower dioxin content.

Technical 2,4,5-T has also been found to be contaminated with a dioxin. In this instance, unfortunately, the very toxic 2,3,7,8 tetrachlorodibenzodioxin,[46-48] was present. The contamination of the 2,4,5-T with 2,3,7,8-tetrachlorodibenzodioxin ranged from < 1 ppm to as much as 32 ppm over the years. In newly manufactured 2,4,5-T in the U.S. the content of tetrachlorodibenzo-p-dioxin has been substantially reduced and is usually kept below 0.1 ppm. Whether other dioxins or furans have ever been detected in 2,4,5-T in the U.S. has not been reported in the literature.

Chlorinated dioxins have also been found in so-called "toxic fat," which produces chick edema disease when fed to chickens. After several years of study by industry and the Food and Drug Administration, Flick et al.[49] were able to separate a purified crystalline product which produced chick edema disease. Two years later, Cantrell et al.[50] announced that 1,2,3,7,8,9-chlorodibenzo-p-dioxin was one of the toxic compounds that produced chick edema. The origin of the chlorinated dibenzodioxin in "toxic fat" was not clear. It was suspected that it arose from chlorophenols which were used to preserve hides for the manufacture of leather. Tallows and greases were obtained from these hides[51] and a more recent outbreak of chick edema disease was traced to the contamination with chlorophenols of soapstock used as feed fats. Higginbotham et al.[52] showed that chlorophenols and their salts, when heated, undergo condensation reactions and form chlorinated derivatives of dibenzo-p-dioxin; furthermore, chlorinated dioxins and predioxins were originally present in some of the chlorinated phenols. Generally no efforts have been made to determine whether *various chlorinated dioxins and furans* are present in the environment. However, Baughman and Meselson[53] recently reported the presence of dioxins in fish taken from four locations in Vietnam in Military Region III, which includes Saigon and several provinces to the north.[54] The concentrations ranged from 18 to 814 ppt.

Zitko[55] was not able to demonstrate chlorinated dibenzodioxins and dibenzofurans in aquatic animals from various locations in Canada.

Chlorinated naphthalenes may so far have remained unidentified since they interfere with other chlorinated compounds in any multiresidue analysis and are detected only when mass spectrometry is also performed.[56]

The various *germicides* given in Figure 2 have been used as antiseptics, deodorants, and particularly, as far as hexachlorophene is concerned, also as preservatives for various cosmetics, soaps, and lotions. A listing of different products that contained hexachlorophene prior to the new regulations was given by the American Pharmaceutical Association.[57] Registration of bithionol was cancelled in the U.S.[58] because it produced photosensitivity reactions in humans using soaps that contained this product. None of the polychlorinated polycyclic compounds used as germicides have been shown to accumulate in the environment. Hexachlorophene has additional uses as a fungicide on ornamental plants, on conveyor chains as a mildewstat, in laundry rinses as a mildewstat, in shoes and air filters and assorted industrial uses as a fungistat, and on leather, paper, and textiles.[59] It is also registered as a pesticide for use on cucumbers, peppers, and tomatoes,[60] and for cotton.

Dichlorophene is used as a mildew preventive on cotton and is recommended as a fungicide and bactericide for the protection of textiles and materials from molds.[61]

CHEMISTRY: METHODS FOR CHEMICAL DETERMINATION AND PHOTOLYSIS

Much of the chemistry of *polychlorinated biphenyls* has recently been reviewed.[33-34] Commercial chlorinated biphenyls are mixtures of biphenyl groups with different numbers of chlorine atoms (Figure 1). When hydrogen atoms are replaced by chlorine on the biphenyl ring, a large number of substitution combinations can arise since the number of chlorine atoms on the molecules can vary and the chlorines can also be

attached to the molecule in different positions, resulting in a variety of isomers. Even if the chlorination may be the same, different commercial batches may vary in the relative concentration of isomers. The PCBs produced in the U.S. by Monsanto fall under the trade name Aroclor. The various Aroclor mixtures have a great number of constituents, and when the biphenyl is chlorinated, 210 different chemicals are theoretically possible. Aroclor 1254, for instance, yielded 69 constituents while the fractionation of Aroclor 1242 and 1260 yielded 45 and 78 components, respectively.[34]

The chlorinated biphenyls (PCBs) are chemically very inert, resistant to corrosive chemicals, insoluble in water, and have a low vapor pressure. Aroclor 1221, for instance, has a boiling point of 278°C. The boiling point increases with increasing chlorination. The chlorinated biphenyls are determined in biological and environmental samples by a combination of electron capture gas liquid chromatography and mass spectrometry. Nuclear magnetic resonance spectroscopy is used in addition to the above two procedures in some laboratories. PCBs were first identified in environmental samples by Jensen in 1966.[62] They were detected as interfering peaks on GLC analysis of environmental samples that were analyzed for chlorinated pesticide residues.[63]

The PCBs interfered with routine pesticide analyses for chlorinated hydrocarbons. Various authors have therefore published separation methods[64] and Reynolds[38] discussed this extensively in a review.

According to Safe and Hutzinger,[65] the use of mass spectrometry for structural studies of PCBs is limited because the chlorine atoms may be randomized between the phenyl groups when the PCBs are fragmented by electrons. The only exception to this is 2,2'-dichlorobiphenyl.

The quantitation of polychlorinated biphenyl in environmental samples has met with difficulty since the PCBs represent mixtures. Several workers have compared PCB components in field samples to Aroclor 1254 because the sample chromatograms were most similar to these mixtures.[66-68] Rote and Murphy[68] quantitated individual peaks of different Aroclors by means of a response curve. The semilogarithmic relationship of detector response (total peak area/16 ng) to average chlorine content was obtained for each Aroclor by the method of least squares. From this relationship the theoretical response of the detector to each chlorinated biphenyl was calculated. When this approach was used, the levels of PCBs were lower than when the PCB levels were compared to the Aroclor 1254 standard. Because of these difficulties in quantitation, the PCB levels reported from different laboratories may vary greatly. The various problems encountered in the analytical methodology of polychlorinated biphenyls and their quantitation are also described in a U.S. FDA report which can be obtained upon request from the U.S. Food and Drug Administration in Rockville, Md. The title of the report is *Analytical Methodology for Polychlorinated Biphenyls* (Feb. 1973).

Polychlorinated terphenyls (Figure 1) are not eluted from gas chromatography columns under conditions used for polychlorinated biphenyls.[38] By altering their gas chromatograph column, Zitko et al.[37] were able to detect polychlorinated terphenyls.

Chlorinated naphthalenes (Figure 1) elute at the same time and cause interference with the determination of chlorinated biphenyls and chlorinated hydrocarbon pesticides in general, unless mass spectrometry is performed to establish the identity of the various chemicals involved.[56]

The methods presently available for the determination of *trichlorocarbanilide* and *other germicides* (Figure 2) are not very well developed. Graber et al.[69] outlined a thin layer chromatography method for the determination of these chemicals in soap and very briefly mentioned other methods that have been employed in the past.

Several methods for the determination of *chlorinated dibenzodioxins* and *chlorinated dibenzofurans* have been described. The goal of these methods is two-fold. First of all, quite a number of technical polychlorinated organic compounds may contain one or several of these compounds[70-72] as contaminants and methods have been developed to determine them in various technical products. Other methods dealt primarily with toxic fats where many attempts were made to isolate and identify the compounds responsible for chick edema disease.[73] Some of these methods were described in the papers that will be cited in connection with the description of chick edema disease in this article.

When preparing the various chlorinated dibenzodioxins and dibenzofurans for toxicity studies in

order to obtain pure products, methods also need to be available for their separation and identification.[74]

One of the problems with tetrachlorodibenzodioxin as well as tetrachlorodibenzofuran is their severe toxicity in the microgram range which will probably not result in an accumulation within the ppm in biological tissues, for instance, for residue analysis. Baughman and Meselson[53] have recently described a method for the determination of these materials, within the ppt range.

Hexachlorophene is made from trichlorophenol. A process for its production was patented by Gump.[75] Additional methods for the production and purification for hexachlorophene have been published and are cited in *Chemical Abstracts.*

Recently several methods[76-79] have been developed for the determination of small amounts of hexachlorophene in blood and biological tissues. Some methods were based on extraction of hexachlorophene with ether or ethanol or a mixture of ether and ethanol. An acetyl or methyl derivative was formed and the hexachlorophene was determined with electron capture gas chromatography.[79] In an earlier method described by Bachmann and Shetlar,[80] simple benzene extraction was used and no derivative of hexachlorophene was made, which led to very variable results. The various advantages and disadvantages of these methods were discussed by Ulsamer.[79] In earlier work my co-workers used ether for extraction and prepared a dimethyl ether derivative of hexachlorophene.[81] Recovery with this method was only about 75% and we have recently altered our method of extraction to obtain better recovery.[82] A method developed by Kabacoff et al.[78] for the determination of hexachlorophene is, according to the authors, also suitable for determination of dichlorophene and Gutenmann and Lisk[83] have described a method for the determination of hexachlorophene in several agricultural products.

The production of hexachlorophene from trichlorophenol proceeds at low temperature and under acidic conditions which makes the production of a chlorinated dibenzodioxin as an impurity highly unlikely.

Since chemicals that are found in the environment are exposed to sunlight, the effect of *UV light* on the compounds under discussion has been given considerable attention. Hustert and Korte[84] synthetized a few PCB isomers, namely, 2,4,6,2′,4′,6′-hexachlorobiphenyl, 2,4,5,2′,4′,6′-hexachlorobiphenyl, 2,4,2′,4′-tetrachlorobiphenyl, 2,5,2′,5′-tetrachlorobiphenyl, and irradiated the various isomers with UV light. A mercury vapor lamp with quartz filters was used as a light source. The UV spectrum of the synthetized chlorinated biphenyls only shows significant absorption below 300 nm. When these compounds were irradiated in a solvent such as hexane, acetone, methanol, or a mixture of methanol and water, dechlorination and polymerization of the compounds was observed. When the PCB isomers were irradiated in perfluorinated dimethylcyclohexane, an inert medium, isomerization, and chlorination dechlorination were observed. These experiments indicate that, under environmental conditions, photolysis products may result with a lower chlorine content but small amounts of higher chlorinated biphenyls may also occur in low concentrations. Safe and Hutzinger[85] reported that the irradiation of 2,4,6,2′,4′,6′-hexachlorobiphenyl at 310 nm resulted in compounds of lower chlorine content, such as di-, tri-, tetra-, and pentachlorobiphenyls. In these investigations hexane and methanol were used as solvents. It is possible that new compounds are formed by loss of chlorine. Rearrangement and condensation may lead to chlorobiphenyls in environmental samples that are not present in commercial PCB mixtures. The 24-hr irradiation of 3,3′,4,4′-tetrachlorobiphenyl, 2,2′,6,6′-tetrachlorobiphenyl, 2,2′,5,5′-tetrachlorobiphenyl dissolved in hexane resulted in about 70% loss of the starting material, while with 2,2′,4,4′,5,5′-hexachlorobiphenyl, only 3.8% remained and less than 1% of 2,2′,3,3′,4,4′,5,5′-octachlorobiphenyl remained. The irradiation of Aroclor 1254 in hexane resulted in a change of the composition of the Aroclor mixture. The gas chromatogram obtained from the irradiated Aroclor showed shorter retention times than the standard Aroclor 1254. The irradiation of Aroclor 1254 in a dioxane water mixture also containing sodium bicarbonate did not lead to the formation of chlorodibenzofurans or hydroxychlorobiphenyls. The compounds that were observed corresponded to PCB molecules to which water was added and a more polar "carboxylic" acid fraction. Irradiation of Aroclor as a thin film with a frequent addition of water led to newly formed hydroxylated compounds and a more polar "carboxylic" fraction. The results obtained from irradiating

PCBs therefore depended greatly on the solvents used and the pH of the solution in addition to the light source used and may result in dechlorination, formation of polymers, hydroxylation, and carboxylic products.[86]

Exposure of various chlorinated dibenzodioxins to summer sunlight or fluorescent UV light with an intensity of roughly 100 $\mu w/cm^2$ in the presence of organic solvents such as methanol led to rapid photolysis of 2,3,7,8-tetrachloro- and 2,7-dichlorodibenzodioxin, while 1,2,3,4,6,7,8,9-octachlorodibenzo-p-dioxin produced a series of chlorinated dioxins with uniformly decreasing chlorine content. Some evidence was also found that reactions other than reductive dechlorination occurred.[87] When 2,3,7,8-tetrachlorodibenzodioxin was spread on a glass plate in a methanol solution and the methanol was allowed to dry, quantitative recovery of the dioxin was possible even after 14 days of UV irradiation. Similar results were obtained when 2,3,7,8-tetrachlorodibenzodioxin was dissolved in methanol, put on a glass plate, covered with soil, and irradiated.[87] These results demonstrated that under favorable environmental conditions, when organic hydrogen donors are present, photolysis will take place. Bare surfaces of soil, concrete, or water are not effective inducers of photolysis. Chlorinated dibenzodioxins could at times prove to be quite persistent. Partial dechlorination may also occur with chlorinated diphenyls or the higher chlorinated dibenzodioxins and may result in less chlorinated compounds.

The possible long lasting presence of the dioxins is also emphasized by Kearney et al.,[88] who recovered 56 and 63% of originally applied tetrachlorodibenzodioxin after one year in Hagerstown and Lakeland, Md. soils.

Tetrachlorosalicylanilide and similar compounds split off chlorine atoms when exposed to sunlight. This is briefly mentioned in connection with photosensitivity reactions of the skin. Shaffer et al.[89] irradiated *hexachlorophene* in absolute ethanol with UV light and obtained a number of dechlorinated bisphenols resulting from loss of chlorine in the ortho and para positions relative to the hydroxy group.

GENERAL TOXICITY AND EFFECT ON REPRODUCTION

Diphenyl itself, without any chlorine atoms, is a fungistatic agent which is used as a preservative for citrus fruits, and is usually impregnated into the wrapping paper. The protected citrus fruit contains a certain amount of biphenyl residue. In different countries the amount allowed as a residue on citrus fruit varies between 70 to 110 ppm of biphenyl. Booth et al.[90] found a reversible nephrotoxic effect of biphenyl on the rat kidney when rats were fed 0.5% or 1% (10,000 ppm biphenyl in the diet). None of these effects was observed on diets containing 0.1% (1000 ppm biphenyl). Deichmann et al.[91] showed that prolonged exposure to air containing diphenyl in high concentrations of 5, 40, and 300 mg/m^3 produced liver and kidney injury as well as bronchopulmonary lesions in mice and rats. In general, diphenyl has always been considered to be relatively nontoxic. In 1969, in Finland, a man who had been intensively exposed to diphenyl for 11 years became ill and died.[92] Diphenyl impregnated wrapping paper had been manufactured in Finland for about 15 years in the plant where the man was employed. The equipment used in the impregnating process was cleaned 5 to 10 times a year with tri- or tetrachloroethylene. The cause of death of the worker in this particular report was not satisfactorily explained and neither were the complaints of 31 other workers engaged in the same factory. Since chlorinated ethylene compounds were used for cleaning, it should be established whether organic solvents containing halogens could lead to the production of toxic substances when they are brought in contact with diphenyls. If this proves to be the case, then care should be taken that this situation is avoided.

The *PCBs* are also not very toxic when given as a single or a few repeated doses to birds and mammals. However, the juvenile pink shrimp is very sensitive to PCBs in water, while trout and catfish can tolerate higher doses; insects are also not very sensitive to PCBs. Pin fish are not quite as sensitive to a single dose, but will die when exposed for 14 to 45 days to 5 ppb of Aroclor 1254.[93] PCBs are of a low order of toxicity to rats and rabbits when given as a single dose. The acute oral toxicity decreases in rats with increasing chlorine content, while this is not as obvious when the dermal toxicity is tested in rabbits.[33] This may be due to poorer absorption of the higher chlorinated compounds. In studies with adult Sherman strain rats, we found that the oral LD_{50} for Aroclor 1254 and 1260 was somewhere in the

range of 4 to 10 g. These two compounds proved to be more toxic in weanling rats. The single oral LD_{50} for weanlings was 1295 mg/kg, and 1315 mg/kg for Aroclor 1254 and 1260, respectively, and the lowest lethal doses were 1000 mg/kg for Aroclor 1254, and 1200 mg/kg for Aroclor 1260. The intravenous LD_{50} for Aroclor 1254 in adult female rats was 358 mg/kg.[94]

A number of short-term studies have been reported where the PCBs were given to animals over a period of one or two weeks. When the PCBs were given in this fashion they were somewhat more toxic than when they were given in a single dose. These studies are reviewed in great detail by Nelson et al.[32] and will not be reported here since they do not give a great deal of additional information.

Vos and Koeman[95] studied the toxicity of three PCB preparations in chickens, Phenoclor DB6, a French product, Clophen A60, a German product, and Aroclor 1260, a product from Monsanto in the U.S. The birds were fed 400 ppm of PCBs in their diets for 60 days; the Phenoclor and Clophen were much more toxic than Aroclor. The birds fed the two European samples died between 12 and 60 days after onset of exposure. The findings at autopsy consisted of hydropericardium, ascites, subcutaneous edema, and liver necrosis. Only 3 of 20 birds fed Aroclor 1260 died during this time with hydropericardium. None of the Aroclor fed birds showed liver necrosis.

With the help of mass spectrometric and microcoulometric analyses, the authors[95] determined that the two European samples were contaminated with tetra- and pentachlorodibenzofurans. As an additional contaminant, chlorinated naphthalenes were found.

Dermal toxicity studies with the three compounds, Clophen, Phenoclor, and Aroclor 1260, in rabbits[96] after two days led to reddening of the skin where the materials were applied. The skin reaction was more pronounced after one week, desquamation of the external epidermal layers of the skin was observed, the hair grew at a reduced rate, the skin became thickened, and prominent transverse wrinkles developed. The test animals showed a gradual loss of weight, and one of the rabbits given Phenoclor and three of those given Clophen died. All but one rabbit showed fluorescence of the liver under UV light, and microscopic changes were also observed in the livers and the treated skin.

Vos and Notenboom-Ram[97] compared the dermal toxicity of the isomer 2,4,5,2',4',5'-hexachlorobiphenyl with that of a PCB mixture (Aroclor 1260) in rabbits. Their dermal application resulted in early microscopic skin lesions in the Aroclor group. The lesions in the 2,4,5,2',4',5'-hexachlorobiphenyl group appeared later and were less severe. Both experimental groups showed liver changes. The liver damage was more pronounced in the animals that had been exposed to hexachlorobiphenyl than in those that had been exposed to the Aroclor.

In inhalation studies with Aroclor 1242 and 1254, rats, mice, rabbits, guinea pigs, and one cat were exposed to PCB vapors five days a week for several weeks. The concentration of Aroclor 1242 vapor was 1.90 to 8.63 μg/l and no ill effects were observed. The exposure of animals to the vapor of Aroclor 1254 in the concentration of 5.4 or 1.5 μg/l produced enlarged livers in rats. An intercurrent respiratory infection in some control as well as experimental rats made it difficult to interpret the results.[98] Since it is now known that the chronic toxicity of PCBs is a more important parameter in establishing their effects than short-term exposures, further studies with lower levels over longer periods of time would give more meaningful information. Fortunately, most of the compounds under discussion have a low vapor pressure so that exposure by inhalation probably does not present a great problem.

A number of reproduction studies in various species have been conducted with PCBs; usually, embryo toxicity was observed but there were no malformations. Heath et al.[99] were unable to demonstrate an effect on *reproduction* with dietary levels of 25 and 50 ppm of Aroclor 1254 in mallards and bobwhites. An increase in eggshell cracking or reduction in shell thickness was also not observed while DDE, which was used as a positive control, induced significant shell thinning and cracking and a reduction in hatching successes in mallards but not in bobwhite.

In field studies with brown pelican shells, it was found that thinning could be best correlated to DDE residues rather than dieldrin or PCBs. This finding indicates, according to Blus et al.,[100] the PCBs are not responsible for eggshell thinning in most birds following environmental exposure.

Peakall[101] fed ringdoves 10 ppm Aroclor 1254

for 6 months. He injected others with 160 mg/kg intraperitoneally for 4 days, and evaluated the ashed eggshell weights of these birds. He found no difference in eggshell thickness between the experimental and the control groups.

In a pheasant reproduction study with Aroclor 1254,[102,103] a significantly greater difference in the number of eggs that were pipped but not hatched was found in the group of hens that received 50 mg of PCBs weekly. Egg production and hatchability was lower in this group, the survival of chickens that did hatch at six weeks of age was reduced, and the ones that did survive were significantly lighter than the controls.

Aroclor 1242 at 10 ppm or 100 ppm and Aroclor 1254 at 100 ppm in the diets of chickens did reduce egg production and hatchability and caused thin eggshells, while Aroclor 1242 at 1 ppm, Aroclor 1254 at 10 ppm, and Aroclor 1260 at 100 ppm did not affect reproduction in chickens.[104] In a rat reproduction study, decreased survival of pups at a dietary level of 100 ppm of either Aroclor 1242 or 1254 was observed. The results obtained from the reproduction studies in birds particularly seem to indicate that the lower chlorinated biphenyls affect reproduction more than the higher chlorinated biphenyls, and it is very important to determine whether the lower chlorinated biphenyls that have been suggested as replacements for presently employed chlorinated biphenyls have an effect on reproduction.

In another rat reproduction study[94] the lowest dose of Aroclor 1254 that affected reproduction was 20 ppm (1.5 mg/kg/day) which manifested itself in a decreased number of litters and less pups per litter. Neither 1 ppm nor 5 ppm of Aroclor 1254 had an effect on reproduction in the Sherman strain rat; however, an exposure of the dams to the Aroclors at 5 ppm or higher increased the liver-to-body weight ratios in weanling rats of both sexes. At 1 ppm the increase in liver weight was observed only in Fla and Flb weanling *male* rats. The dietary levels of 100, 20, and 5 ppm of Aroclor 1260 did not affect reproduction; however, the liver-to-body weight ratio of 21-day-old pups was increased at all exposure levels.

Mink are highly susceptible to the toxic effects of PCBs. A daily dietary intake of 30 ppm resulted in death in about 6 months. Exposure to 5 ppm Aroclor 1254 in the daily diet severely affected reproduction.[105]

Unfortunately, it is often very difficult to compare the various dietary levels that affect different species, since the food consumption may vary decisively from one animal species to another and the daily food intake in g/kg body wt. is often not included in the scientific report. Some mice strains, for instance, will be exposed to as much of a given substance that is present in the diet at the concentration of 300 ppm as rats on a diet containing 600 ppm or more. With some species it is of course difficult or almost impossible to measure the food consumption, but a greater effort in establishing these very basic facts would be helpful for the interpretation of toxic effects in different species.

Not very much is known at present about the toxicity of *terphenyls* or *chlorinated terphenyls*. These materials have not been studied to any great extent. The few studies that are available only describe very specific effects such as microsomal enzyme induction in the liver and give no overall evaluation of their toxicity.

The most toxic of the various *chlorinated dibenzodioxins* and *dibenzofurans* is 2,3,7,8-tetrachlorodibenzodioxin which, in the German literature, is sometimes referred to as 2,3,6,7-tetrachlorodibenzodioxin. Single oral LD_{50} values are female rats: 44.7 μg/kg; male rats: 22.5 μg/kg; male guinea pigs: 0.72 μg/kg; rabbits: < 30 μg/kg; and dogs: between 30 and 300 μg/kg.[106] It is possible that young animals are more susceptible to the toxic effects of this compound than older animals and LD_{50} values should therefore also be determined in weanling animals. One of the characteristics of this compound was that it caused delayed death, sometimes as delayed as 40 days after a single dose. During the period of intoxication, the animals lost a great deal of weight.[106]

On the other hand, 2.0 g/kg of dichlorodibenzo-p-dioxin and 4.0 g/kg body weight octachlorodibenzo-p-dioxin were not lethal to rats. Hexa-, hepta-, and trichlorodibenzo-p-dioxin were more toxic.[106]

Schulz[107] in earlier experiments found that oral doses of 20 to 50 μg/kg/body weight of 2,3,7,8-tetrachlorodibenzodioxin resulted in fatal liver necrosis in rabbits. More recently Milnes[108] reported that 10 μg/kg was lethal to rabbits.

The 2,3,7,8-tetrachlorodibenzodioxin has a variety of specific toxic effects. Whether these effects vary in different species is presently not well defined. With the interest in this and the other related compounds, more information on

their toxicity probably will soon become available. The problems inherent in studying these compounds are the unavailability of some of them, the fact that they may not be completely pure, particularly the hex- and hepta- preparations, and of course with respect to the tetrachlorodibenzodioxin, its extreme toxicity, which makes it difficult to handle.

In contrast to PCBs, teratogenic as well as fetotoxic effects have been reported for 2,3,7,8-tetrachlorodibenzodioxin. Sparschu et al.[109] gave 0, 0.03, 0.125, 0.5, 2.0, and 8.0 μg/kg/day of 2,3,7,8-tetrachlorodibenzo-p-dioxin to pregnant rats on days 6 to 15 of pregnancy. With the 0.03 μg/kg dosage level, an effect on the fetus was not observed, but doses from 0.125 μg/kg on up resulted in fetal mortality, early and late resorptions, and fetal intestinal hemorrhage. Khera and Ruddick[110] gave doses ranging from 16 to 0.125 μg/kg to Wistar rats on days 6 to 15 of pregnancy. These authors also observed pronounced fetotoxicity at all dosage levels except 0.125 μg/kg. The affected fetuses showed cerebral and intestinal hemorrhage as well as subcutaneous edema at autopsy. The offspring of mothers given 0.5 and 1 μg/kg of the tetrachlorodibenzo-p-dioxin gained less weight during their suckling period and their survival to weaning was reduced. This could have been caused by the intrauterine effect of the dioxin or by excretion of the dioxin in the milk of the dams given the material during pregnancy. Courtney and Moore[111] observed cleft palates in 3 strains of mice given 3 μg/kg TCDD subcutaneously from days 6 to 15 of pregnancy and an increased incidence of kidney anomalies. Hydronephrotic kidneys were also produced in mouse pups that were nursed by dams treated with TCDD during pregnancy or at time of parturition.[112]

The 2,3,7,8-tetrachlorodibenzo-p-dioxin is extremely toxic in the chick embryo assay.[52] In the rat, hexachlorodibenzodioxin caused fetotoxic effects at 1 and 10 μg/kg and teratogenic effects at doses of 100 μg/kg.[106] Harris et al.[113] gave female rats daily doses of 0.1, 1, or 10 μg/kg TCDD for 31 days. Rats given 10 μg/kg lost weight and most of them were moribund within 3 weeks. Weight gain was also less at the 1 μg/kg dietary level. The rats receiving 0.1 μg/kg were not affected. In experiments with guinea pigs, 9 of 10 animals died after a single dose of 3 μg/kg TCDD, while guinea pigs receiving a single dose of 1 μg/kg gained less weight. All guinea pigs receiving 1 μg/kg/week died within 24 to 32 days and a weekly dose of 0.2 μg/kg depressed their weight. Of the various animal species tested, the guinea pig seemed to be most susceptible to the toxic effects of TCDD..

Reduced thymus and spleen weights were observed in rats that received a single dose of 25 μg/kg TCDD. The rats that died at the various dosage levels showed massive hemorrhages into the heart, liver, brain, adrenal glands, and gastrointestinal tract. Necrosis and ulceration of the glandular part of the stomach were observed. Occasionally organized thrombi were noted and the liver revealed necrosis of parenchymal cells. Sublethal doses of TCDD produced microscopic changes in liver, kidneys, and thyroid. In the kidneys the collecting tubules were primarily affected. Doses of 0.1 μg/kg/day for 31 days, a single dose of 5 μg/kg, and multiple weekly doses of 1.0 and 0.2 μg/kg did not produce changes in rats discernible with the light microscope.

Toxicity studies were conducted in our laboratory with *dichlorophene*.[114] The oral LD_{50} for adult female rats when the material was given in peanut oil by stomach tube was 1,660 mg/kg and in males it was 1,500 mg/kg. The only signs of toxicity observed in these rats were diarrhea and some depression. When 200 mg/kg was applied daily in propylene glycol to the skin of a clipped area of the back of male adult rats as a 12.5% solution, local irritation of the skin with some ulceration resulted but there was no systemic toxicity. The acute intravenous LD_{50} in adult male rats was 16.8 mg/kg when it was given in a saline lecithin suspension. In a two generation rat reproduction study, a dietary level as high as 1,000 ppm (50 mg/kg/day) had no effect. Gross and microscopic examination of the various organs including the brains of the parents as well as the offspring did not reveal any morphological changes that could be related to dichlorophene exposure. These findings indicated that dichlorophene was not very toxic to rats. The great difference between the oral and the intravenous LD_{50} suggested either poor absorption or rapid breakdown of the material in the gastrointestinal tract. Martins[61] lists the acute LD_{50} for guinea pigs as 1,250 mg/kg and for dogs, 2,000 mg/kg. Rats fed for 90 days on a diet containing 2,000 ppm showed no evidence of toxicity.

The acute toxicity of *hexachlorophene* is much greater and varies in different animal species and is

also dependent on the route of administration and the solvent in which it is given. Gump[115] lists the acute oral LD_{50} in mice as 168 mg/kg and cites LD_{50} values reported by other investigators ranging from 80 mg/kg to 215 mg/kg. Dogs and sheep were more susceptible to the toxic effects of hexachlorophene. The interest in the effect of hexachlorophene on sheep in particular stemmed from the fact that it is effective against liver flukes[116] and advocated for its anthelmintic properties. This even led to the treatment of children with orally administered hexachlorophene in China.[117] Intravenously administered hexachlorophene in rats resulted in an LD_{50} value of 9.1 mg/kg and in rabbits of 8.5 mg/kg.

More recently we found that the oral LD_{50} (single dose) for adult male rats was 66 mg/kg and for adult female rats 56 mg/kg; for weanlings it was 120 mg/kg when the material was given in peanut oil by stomach tube to Sherman strain rats. The acute intravenous LD_{50} was 7.5 mg/kg in adult male rats.[118] Nakaue et al.[119] reported similar oral LD_{50} values in Wistar rats. The single oral LD_{50} for sheep and cattle lies between 30 and 60 mg/kg.[120]

Single dermal LD_{50} values have not been reported in the literature. We found in rats[118] that single applications of 600 mg/kg hexachlorophene in 95% ethyl alcohol solution caused death in 1 of 10 rats. Dermal application of 24 and 48 mg/kg/day for 30 days in propylene glycol or in a detergent as a 3% solution caused ulceration of the skin and affected the white matter of the central nervous system in at least half of the rats. A dose of 12 mg/kg/day as a 1.5% solution caused only mild erythema and desquamation of the skin of rats. No effect on the central nervous system was observed. Whether a single dose given in either propylene glycol or a detergent would be more toxic than one given in ethyl alcohol was not established.

The signs of acute hexachlorophene poisoning following a single oral dose varied with the age of the rats.[118] Adult rats suffered from severe depression and diarrhea, but showed few signs of leg weakness. Weanlings, on the other hand, developed posterior leg weakness as well as depression. The leg weakness became more apparent in the adults when they were given repeated doses of hexachlorophene. Death was usually preceded by convulsions in rats dosed intravenously. In rats killed with hexachlorophene, rigor mortis developed very rapidly and was very pronounced. Body temperature elevation following hexachlorophene poisoning has also been recorded in rats.[119] Both changes, the pronounced rigor mortis and the temperature elevation, may be related to the fact that hexachlorophene uncouples oxidative phosphorylation. Additional symptoms of hexachlorophene poisoning particularly in other species are discussed under neurotoxicity.

Nakaue et al.[119] fed 400 ppm hexachlorophene (28.9 mg/kg/day) to Wistar rats. They developed leg weakness within 5 days, diarrhea, emaciation, and they died in 7 to 10 days. No deaths occurred in the rats that were fed 200 ppm (about 13 mg/kg/day) for 16 weeks.

In a two generation rat reproduction study which we conducted in our laboratory, we found a reduced survival in the offspring of the F_1 generation fed 100 ppm (11.8 to 5.5 mg/kg/day) in their diet for 54 and 166 days. This was statistically significant in the second breeding of the F_1 generation. In the second generation the litters were slightly smaller, but the survival to weaning was not significantly reduced.[118] A dietary level of 20 ppm (2.3 to 1.1 mg/kg) did not influence reproduction. Thorpe[121] reported that the oral administration of hexachlorophene to male rats as well as single doses of 25 and 50 mg/kg hexachlorophene to sheep produced degeneration of spermatogenic cells; multinucleated cells were observed within the tubules of the testes. The doses given by Thorpe were higher than the ones we used in the reproduction study, and microscopic examination of the testes of the rats used in our reproduction study did not reveal any abnormalities. Toxic doses of hexachlorophene given to the dam during pregnancy may result in malformations of the offspring. Thus far, two studies have been reported in the literature; both studies were conducted in rats.[122,123] It should be determined whether malformations can also be produced in other species and with lower doses before any conclusion can be drawn from these findings.

A number of *human* poisoning cases have been reported in the literature and some of these were fatal. Most of the poisoning cases in humans have either been caused by accidental ingestion, because a hexachlorophene detergent solution was mistaken for milk of magnesia, or by the application of hexachlorophene to large areas of burned or otherwise damaged skin.[115,124] Larson[3] was

the first to recognize that burned patients developed convulsions and other toxic symptoms after repeated exposure to hexachlorophene which was applied to burned areas of the skin. The incidence of convulsions in burned patients decreased when the use of hexachlorophene was discontinued.[125]

Eleven cases have been reported[126,127] in which a single dose of 3% hexachlorophene detergent solution had been taken orally. Gastrointestinal symptoms including nausea and vomiting were observed in ten of these cases; the eleventh case, a child who drank about 250 mg/kg hexachlorophene, became comatose and died. One additional death occurred in this group, however, this was most likely due to other causes. A 17-day-old infant was accidentally given daily oral doses of 37 mg/kg hexachlorophene for 7 days. On the third day the child started to have loose stools. On the fourth day of hexachlorophene administration the child developed spasms of the extremities with twitching of the face, sucking movements of the lips, and lateral shaking movements of the head. It was also noted in the case report that the child refused to take the medicine, there was drooling and frequent vomiting, and it is not quite clear whether the child received 37 mg/kg/day. The child survived. A week after the hexachlorophene suspension was discontinued, persistent spastic flexion of the fingers, hands, and arms was still observed. After a month the hands were slightly flexed at the wrists but were not spastic. Horizontal nystagmus was observed but cleared later. The child was discharged when it was two months and three weeks old. At this point it had shown great improvement and when checked at six months the child was healthy looking.[128] Pilapil mentioned in his discussion a number of other unpublished cases, some of which were reported to the National Clearing House for Poison Control Centers. Among these was the case of an infant who by mistake got a spoonful of PhisoHex in each feeding bottle for approximately two weeks, when it suddenly died.

Chung et al.[117] treated a total of 105 patients 5 to 15 years old with hexachlorophene for chlonorchiasis sinensis infestation. Sixty of these patients received 1 dose of 20 mg/kg, 32 received 2 doses of 20 mg/kg/day, 8 patients received 3 daily doses of 20 mg/kg, and 5 patients received 20 mg/kg body weight on the first day and 10 mg/kg on the second day. The toxicity reactions observed in these patients were mainly confined to the central nervous system and to the gastrointestinal tract. The group that received 3 doses of 20 mg/kg/day had the most severe reaction. One patient in this group became comatose on the fourth day with loss of light reflex and positive pyramidal signs. Fundus examination of the eyes revealed mild papillary edema. A low grade fever was also observed. Complete recovery occurred within three days. One half to two thirds of the patients had diarrhea within 1 to 2 hr after medication and in some cases a marked depressive effect on the central nervous system was also observed. An additional case of poisoning after oral ingestion was reported by Korlof and Winsten[129] in Scandinavia and two fairly recent cases with fatal outcome occurred in the U.S.[130]

Herter[131] was probably the first to report a human poisoning case caused by the dermal absorption of hexachlorophene. Because of a language barrier, the mother of a newborn infant applied 3% hexachlorophene as a lotion after the infant's bath without rinsing the baby. After four days, excoriations appeared on the child's face and buttocks, the infant developed convulsions, his face twitched and his extremities jerked. A roving nystagmus was present. This child recovered within 16 days after exposure to hexachlorophene had been discontinued.

In addition to the two cases already mentioned in which poisoning occurred because of oral ingestion, Mullick[130] also reported four cases – children who died following the treatment for burns in two instances and the treatment for severe ichthiosis with a 3% hexachlorophene bath.

Larson[3] reported six burned patients who developed central nervous system symptoms following the dermal exposure to hexachlorophene. All six patients recovered. Korlof and Winsten[129] reported two cases of poisoning following the dermal application of hexachlorophene. Both patients were fairly extensively burned, and developed central and peripheral nerve damage as well as some kidney impairment.

Lockhart[132] mentioned six deaths that were reported to the FDA caused by the topical application of hexachlorophene on burned patients. In all of these cases the skin was damaged, probably enhancing absorption of hexachlorophene, and although skin does represent a barrier, it is not impermeable – a fact we tend to forget at times. Damaged or abnormal skin may be

more permeable, and the skin of prematures, newborns, and children in general may have absorption characteristics other than those of adult human beings, another factor that is often neglected. Recently[133,134] 41 deaths due to hexachlorophene poisoning have been reported in France. The deaths occurred in infants who were treated with a powder to which 6% hexachlorophene had been added by mistake. This powder was mainly applied in the diaper area where the skin became inflamed, this as well as the diaper which served as an occlusive dressing, probably enhanced absorption. These cases will not be made public until litigation problems have been settled.

Little published information is available on the toxicity of the germicides in Figure 2 aside from hexachlorophene and dichlorophene. The acute oral LD_{50} for triclosan (TCC) in mice and rats is about 4,000 mg/kg.[135] The acute subcutaneous toxicity for rats was 14,700 mg/kg and the acute dermal toxicity was about 9.3 g in rabbits. Concentrations of 1 to 5% solutions caused eye irritation in rabbits. Daily oral doses of TCC up to 1,000 mg/kg/day for 4 weeks did not produce an effect in rats. When 2.5 and 5% suspensions in gum arabic were applied dermally to rats for 4 weeks, the animals did not gain as much weight. Unpublished material cited in the minutes of the OTC (Over the Counter Drugs) Panel listed a "no effect" level as 170 mg/kg/day in rats and for a 90-day feeding study in rabbits, 138 mg/kg/day. In baboons a daily oral dose of 100 mg/kg/day did not produce a toxic reaction. The minutes also mentioned some work where lower doses showed an effect on rats and dogs. Since some of these results are conflicting, further studies are indicated and should be performed with products used as ingredients in soaps; a chemical analysis of these products is necessary to determine whether impurities might be responsible for varying results. Chloroaniline as a result of heating during manufacture could be present as such an impurity. Of the anilines, according to Hamblin,[136] only p- and m-chloroaniline may cause methemoglobinemia if absorbed through the skin. The o- and m-chloroaniline may cause liver and kidney damage. Triclosan itself is also absorbed through the skin and absorption is greater when applied to damaged skin.

SPECIAL TOXIC EFFECTS

Skin Reactions

Chloracne

This occupational skin disease can be produced by a number of chemical compounds.[137] Polychlorinated biphenyls as well as the chlorinated naphthalenes, a few isomers of the chlorinated dibenzodioxins, and chlorinated dibenzofurans are all able to produce chloracne in a certain proportion of the human population. Oily skin and large pores seem to predispose to the disease while the opposite is true for smooth, tender skin and it is possible that young children might be less susceptible because of the nature of their skin.

Chloracne has also occurred in workers engaged in the production of 2,4,5-T[1]. During the industrial production of trichlorophenol a sudden rise in temperature within the reactor has occasionally resulted in accidents. In one instance this exothermic reaction caused an explosion.[108] When the rise in temperature occurred, 2,3,7,8-tetrachlorodibenzodioxin was formed and severe outbreaks of chloracne were observed among the workers.[137a]

The term chloracne was first used by Herxheimer in 1899,[138] who thought it was caused by free chlorine generated in certain factories. Wauer in 1918[139] and Teleky in 1927[140] suggested the term "Pernakrankheit." Another term sometimes used is halogenwaxacne.

As the word chloracne implies, part of the lesion of this occupational skin disease resembles adolescent acne; however, it is generally more severe and the distribution of the lesions is not consistent with adolescent acne, although it may be superimposed on adolescent acne. Chloracne is described in the literature as consisting of the formation of comedones with or without cysts and pustules. The follicular orifices are filled with sebaceous and keratinous material. Melanosis and a secondary inflammatory reaction may also exist. In addition to this description, skin changes occur that have been discussed less frequently in the more recent literature. These skin changes are usually referred to as cable rash or cable itch. Cable rash has been observed in workers who have insulated cables with various mixtures of chlorinated naphthalenes and chlorinated biphenyls and in workers handling these cables.[141] It has been observed also in factories that make condensers.[142,143] According to Braun,[142] a few of

the cases with manifest chloracne started with changes in the skin of the face resembling photo sensitivity and the bearers of these lesions suffered severe puritus in the areas of the skin lesions. This lesion termed cable rash occurred predominantly on the cheeks but also on the skin of the arms. The outcome of cable rash varied; it could either disappear or it could develop into full-blown chloracne.

In addition to the pustules and comedones of typical chloracne, brownish keratinization of the skin was observed in some patients. Braun[142] pointed out that the workers, particularly those with poor hygene, transmitted the disease to their spouses and children through direct contact.

Many of the occupational cases of chloracne that have been reported in the literature have been reviewed previously.[137,144] Jones and Alden[145] reported one of the first outbreaks of chloracne in a company that was engaged in the manufacture of chlorinated biphenyls. They descrioed one typical case and mentioned 24 men working on the manufacture of chlorinated biphenyls; 23 had had an acneform eruption on the face and body. Particularly during the 1930s and 1940s large outbreaks of chloracne occurred in a number of factories.

Much discussion has been devoted in the literature to the problem of whether the chloracne was produced by external contact alone or whether it was caused by systemic absorption, particularly the inhalation of vapors. One question asked was whether internal administration could cause chloracne, and if so what part it played in any particular case. As we know now from outbreaks of poisoning due to the consumption of rice oil that was contaminated with polychlorinated biphenyls in Japan (see under "Yusho"), chloracne can be produced by the systemic absorption of chlorinated biphenyls. The experiments by Shelley and Kligman[146] and Plewig[147] showed that the skin lesions could also be produced by applying the chloracnegenic compound to the skin.

After occupational chloracne manifested itself, the patients also complained of systemic effects such as loss of appetite, nausea, edema of the face and hands, abdominal pain, vomiting, and burning and soreness of the eyes. Particularly with the chlorinated naphthalenes, hepatotoxic effects have also been observed. Application of chemicals to the skin can lead to their absorption and cause systemic toxicity. Whether a systemic toxic effect results will depend on the skin surface area covered, the amount of the toxic substance applied, and the ability of the skin to absorb it. If dermal absorption is poor, a single application will usually not be very toxic. However, if the compound is not easily metabolized and excreted, repeated dermal application may result in sufficient accumulation in the body to cause systemic toxicity.

Crowe[144] has pointed out that chloracne due to chlorinated naphthalenes has decreased because these compounds have been used less since the Second World War. However, Weber in 1969[148] reported an outbreak of chloracne in a company engaged in the production of electric coils that were coated with a mixture of chlorinated naphthalenes with the tradename "Nibrenwax."

Detailed examination by several German investigators indicated that the capacity of 2,4,5-trichlorophenol as well as 2,4,5-T (2,4,5-trichlorophenoxyacetic acid)[149,150] to produce chloracne was due to contamination with 2,3,7,8-tetrachlorodibenzofuran or 2,3,7,8-tetrachlorodibenzodioxin.

Schwartz et al.[151] indicated that the most potent chloracne producing agents were the chlorinated naphthalenes, chlorodiphenyls, and chlorodiphenyloxides. Persons who worked with chlorinated naphthalenes usually developed acne after a month or more of exposure.

Light microscopic observations made of the chloracne lesions of humans varied somewhat, depending primarily on the length of time the lesions had existed at the time of examination. The earliest change encountered consisted of acanthotic widening of the external root sheath of the hair follicle. Above a layer of basal cells 6 to 10 layers of large round cells were observed, followed by a layer of 2 to 3 cell rows of granular cells. In later skin biopsies, folding of the basal layer occurred and comedo formation was observed, resulting in dilatation of the follicle and atrophy of the epithelium surrounding the comedo. The contents of the follicles consisted predominantly of keratinous material rather than sebum. The sebaceous glands also underwent changes. Within the alveoli of the glands the basophil cells increased in number and basal cell hyperplasia was noted. The ducts of the sebaceous glands were filled with a solid mass of basophil epithelial cells. The sebaceous glands gradually

disappeared and in later secretions were only recognized as solid protrusions of the comedo wall. Aside from atrophy of the sebaceous glands, hyperplasia has also been described and many large sebaceous glands forming huge cysts filled primarily with keratinous material have been observed. Hyperkeratosis and acanthosis of the surrounding epidermis has usually accompanied these lesions. Foreign body granulomata which are thought to result from rupture of the follicular cysts have been found in the skin.[147,151] In the Japanese poisoning incident (Yusho), the predominant skin lesion was marked hyperkeratosis of the epidermis, cystic dilatation of the hair follicles, and an increase in the melanin pigment in the basal cells of the epidermis. Foreign body granulomata were also observed. The contents of the cysts consisted partially of keratin. In a stillborn child the hyperkeratosis was even more pronounced, accompanied by atrophy of the epidermis; cystic dilation of hair follicles was observed, especially in the skin of the head. According to earlier reports[151] some differences exist in the microscopic changes observed in the skin following exposure to the various chlorinated cyclic compounds that can produce the lesion. It is, of course, possible that these histopathologic variations are in reality only differences that exist between an early, a well-developed, and a subsiding lesion.

Many theories have been advanced to explain the development of chloracne. None is satisfactory. The concept of mechanical plugging of the hair follicles on skin exposure to polychlorinated polycyclic compounds is not supported by the fact that chloracne develops following ingestion of these compounds. It is possible that the skin lesions are caused by a toxic effect on the epithelium of the skin and its appendages which is manifested in proliferation and loss of normal function of the cell. Since the chemicals are lipid soluble they could occur in a more concentrated form in sebum and therefore affect sebaceous glands more than sweat glands or salivary glands. The reports on the Yusho outbreak suggest that proliferation of the epithelium of the mucous membranes may also occur. The increased pigmentation of the skin and mucous membranes may indicate a functional disturbance resulting in increased melanin production. Hyperpigmentation is also observed when the adrenals are damaged, as in Addison's disease, and adrenal function should be investigated in chloracne patients who manifest hyperpigmentation.

In none of the human case reports on chloracne have the mucous membranes been studied to any great extent. Vaginal smears in women and sputa, corneal, and nasal scrapings may give helpful information in this respect. Since in animals exposed to polychlorinated polycyclic compounds low vitamin A levels have been observed in the liver, it is possible that the compounds may affect vitamin A absorption and storage, and adequate utilization in the liver and elsewhere in the body. If the lesion is produced by inadequate utilization of vitamin A, then administration of vitamin A per se may not necessarily alter the course of the disease. Protein deficiency may also mimic hypovitaminosis A in that it affects transport of vitamin A. The liver stores 90% of the available vitamin A and these stores may be affected in liver disease. The microscopic appearance of the skin lesions resembles in some respects observations made in skin of patients suffering from vitamin A deficiency. In vitamin A deficiency, hyperkeratosis of the hair follicles is observed. Multiple firm papules may develop as a result of the keratin plugs in the sebaceous glands. Dryness and scaliness as well as furunculosis of the skin may also be present. In contrast, night blindness has not been reported as a symptom in patients suffering from chloracne. Unfortunately, chloracne outbreaks are often not well investigated and, because of problems with litigation, accounts are often only published many years after the outbreak has occurred if they are published at all.

Chloracne is a very persistent skin disease and in the Japanese outbreak of poisoning with chlorodiphenyls, the skin changes were still present in a number of patients three years after exposure to the chlorodiphenyls had been discontinued. What needs to be investigated is whether this persistence of the skin disease is caused only by the fact that once the lesion has developed it regresses very slowly, or whether it may be sustained by the chloracne-producing chemicals which have been absorbed and stored in the body, particularly the adipose tissue, and are only very gradually excreted. In patients with chloracne, adipose tissue biopsies should be analyzed for the presence of the offending polycyclic polychlorinated compounds. If adipose tissue is unobtainable, the sebum of the ear canal or the content of the skin lesions may represent a good substitute.

Apparently there is no known effective treatment for chloracne and the best control measure is prevention. The manufacture of chlorinated hydrocarbons and the coating of wires and condensors with insulating materials containing them should be done in totally enclosed areas so that the fumes of the insulating substances and of the solvents if any are used do not come in contact with the workers. The workers should be provided with clean overalls and underclothes daily. These should be laundered at the plant and cleaned in such a way that no chlorinated hydrocarbons remain on them. Shower baths after work should be compulsory and supervised and there should be sufficient shower facilities provided so that the workers are not unduly delayed after work. Special synthetic wetting agents[151] seem to remove the material from the skin more effectively than ordinary soaps.

The experimental induction of chloracne in men has also been accomplished with 2 applications of 10 μg 2,3,7,8-tetrachlorodibenzo-p-dioxin[107] and with Halowax 1014, pentachloronaphthalene, and hexachloronaphthalene.[147]

Experimental work in animals has shown that most species do not develop the same type of dermatitis. The rabbit's skin,[152] particularly the inner aspect of the ear lobe, provides a good test medium for the development of acneform dermatitis. The rabbits, in addition to the development of hyperkeratosis of the ear, will also show a hepatotoxic effect.[150] Microscopic examination of the changes in the rabbit ear were described by Jones and Krizek.[153] Inagami and Koga[154] were able to produce hyperkeratosis in hairless mice that were fed rice oil contaminated with Kaneclor 400. These mice also showed hepatomegaly. Microscopic examination of the skin revealed cystic dilation and hyperkeratosis of hair follicles and sweat glands. Hairless mice may be another suitable experimental animal for testing the effect of these chemicals on the skin.

X-disease in Animals

Chloracne-type lesions do not usually occur in other species except for the rabbit and the hairless mouse, already mentioned. However, in 1947 Olafson[155] described a disease in cattle which was designated as *X-disease* or hyperkeratosis. Symptomatology included excessive lacrimation, diarrhea, polyuria, marked salivation, and discharge from the nostrils. The animals developed a chronic cough, poor appetite, numerous red maculae in the buccal mucosa; in addition, hyperkeratosis of the skin developed. The skin was hard, and fissured particularly across the withers and sides of the neck. Many wart-like proliferations on the lips, tongue, and hard palate were also observed. The abomasum was greatly inflamed, swollen, and edematous with many superficial ulcers.[156] In the cows that had X-disease, a low vitamin A plasma level was also observed, which again indicates that vitamin A may play a role in the etiology of these various disease entities.

The disease was first thought to represent a virus infection and other factors were also suspected. After numerous investigations it was finally established that X-disease could be produced in cattle by the ingestion of highly chlorinated naphthalenes, and also by petroleum products such as crankcase oil. Bell[157] tested the ability of the various compounds of the chlorinated naphthalene group to produce X-disease. He demonstrated in his studies that dichlorinated and trichlorinated naphthalenes did not result in X-disease, while tetrachloronaphthalene had an effect and the higher chlorinated naphthalenes such as pentachloronaphthalene, hexachloronaphthalene, heptachloronaphthalene, and octachloronaphthalene caused severe disease. Octachloronaphthalene was less toxic than hexa- and heptachloronaphthalene. Wagener[158] reported the occurrence of hyperkeratosis in cattle in Germany between 1946 and 1948. The cause of this outbreak was traced to a wood preservative which was used in postwar Germany. The author proved experimentally that animals housed in cabins painted with the wood preservative developed typical X-disease. The poisonous ingredient in the wood preservative appeared only in certain lots of this product and later lots did not contain it. The poisonous ingredient was never identified by chemical analysis.

Animal experiments were conducted with the toxic ingredients of rice bran oil that produced Yusho in Japan. These toxic substances consisted of a mixture of chlorinated biphenyls containing 48% chlorine, with a trace of 0.01% of naphthalenes.[159] When mice were exposed to them for 3 to 4 months, they showed eczematous changes of the skin around the eyelids, erosion, ulceration and perforation of the ear laps, loss of hair, and erosion and ulceration of the skin around the neck, forelegs, and sides of the chest. The authors

stated that, at the end of the experiment, some changes in the skin similar to those in the experimental mice were seen in some of the control mice also, which makes it difficult to interpret their findings.

In 1957 a disease that will be discussed in greater detail later in this article occurred in a large number of chickens. It was termed chick edema disease and was found to be caused by certain toxic fat. Allen and Carstens[160] fed this substance to monkeys and found that, among other changes which will be discussed later, the monkeys developed a generalized alopecia and subcutaneous edema one to two months before death. Schoettle et al.[161] produced hyperkeratosis of the skin in rats by feeding them hexachloronaphthalene. Young swine[162] and sheep[163] did not develop skin lesions as a result of exposure to chlorinated naphthalenes.

Photocontact Dermatitis and Irritation of the Skin

As was pointed out earlier, the hyperpigmentation observed in patients with chloracne as well as the reaction described as cable rash could also be the result of a photosensitive reaction. This photosensitive reaction is the predominant lesion in the skin produced by a number of germicides, namely, bithionol (Figure 2), tetrachlorosalicylanilide (TCSA), and a number of other halogenated phenolic compounds including fenticlor. Patients who develop photodermatitis complain initially of a burning sensation, and this is followed by itching. A rash develops which in the beginning is erythematous and associated with varying degrees of edema. As it subsides, pigmentation is observed.[164] Wilkinson[165] was the first one to describe cases where patients developed a photo allergy which was due to the incorporation of TCSA into popular soaps. He observed that the outbreak of dermatitis was confined to the areas that usually were exposed to light. Most of the cases reported from England were transient and as soon as exposure to the compound was discontinued the reaction subsided. Jillson and Baughman[166] and Jillson,[167] on the other hand, reported that their cases were more persistent.

Willis and Kligman[168] demonstrated that the photocontact allergy of the skin with these various compounds was simply a contact sensitization. The reason that allergic reactions of the skin developed only after exposure to the sun was due to the photodecomposition of the various products to which the skin had been exposed. If various brominated and chlorinated salicylanilides were irradiated in vitro in an appropriate solvent, the resulting breakdown products caused an allergic reaction on contact, without exposure to UV light. These studies were performed because of information provided in earlier studies by Coxon et al.,[169] who had irradiated brominated and chlorinated salicylanilides and found that these compounds gave off halogens. With 3,5 substituted salicylanilides, the halogen substituted on the 3 position was lost. Tetrachlorosalicylanilide photosensitized subjects reacted to 3′, 4′, 5-trichlorosalicylanilide and to 3′, 4′-dichlorosalicylanilide. A reaction to nonhalogenated salicylanilide was not observed. Halogens on the anilide ring were unaffected by UV radiation, while the halogens on the salicyl ring were replaced stepwise by hydrogen upon irradiation.

Among the patients who developed photosensitivity to tetrachlorosalicylanilide reported by Wilkinson,[165] a group of patients in a shop of a factory was also included. This same outbreak was reported by Jones,[170] who found that among 106 employees who used the same soap very frequently, 31% were affected by a dermatitis due to contact with tetrachlorosalicylanilide present in the soap.

Additional cases of photodermatitis due to the exposure to tetrachlorosalicylanilide were reported by Calnan et al.,[171] who pointed out that pronounced edema of the eyelids was also observed when involvement was severe. Some cases were persistent or developed acute relapses on further exposure to sunlight without further exposure to the offending chemical. Frenk[172] reported an additional nine cases of acute contact dermatitis of the hands due to tetrachlorosalicylanilide. Epstein[173] described a photoallergic reaction in two patients to tribromosalicylanilide (TBS). With patch tests these patients showed a cross reaction to TCSA. A similar cross reaction pattern to a number of halogenated salicylanilides was also observed by Osmundsen[174] in patients who had developed photodermatitis following exposure to tribromosalicylanilide.

Because of the photosensitivity reactions and the cross reactions that were also found with bithionol, which is similar in structure to tetrachlorosalicylanilide (Figure 2), the U.S. Food and Drug Administration, on October 24, 1967, withdrew all new drug applications of all drugs that

contained bithionol. In the notice of withdrawal of approval in the Federal Register, the Commissioner of the Food and Drug Administration pointed out that bithionol may in some instances cause a very persistent photosensitization and severe dermatitis may occur with exposure to sunlight without further contact with the sensitizing articles, and that bithionol may produce cross photosensitization with other commonly used chemicals such as certain halogenated salicylanilides and hexachlorophene.

Hexachlorophene preparations may also cause irritation of the skin if these preparations are used frequently. A true allergic reaction to hexachlorophene is apparently rare.[175] The more severe reactions were usually confined to the skin of the scrotum and consisted of a primary irritant contact dermatitis.[176,177] The cases reported by Baker et al.[176] also included a 33-month-old Caucasian female who developed a rash around the buttocks, vulva, and upper thighs following the use of a hexachlorophene preparation in her bath water.

We observed severe skin irritation and ulceration in rats that were exposed dermally to a 3% hexachlorophene solution in propylene glycol or a detergent. The skin reaction was not as severe when the hexachlorophene concentration was reduced to 1.5%.[118] Boutwell et al.[178] reported considerable skin irritation in mice that had 5 mg/mouse applied twice weekly for 21 weeks. Harber et al.[179] were able to induce contact photosensitivity with TCSA or TBS. These authors also found that cross contact sensitivity and photosensitivity to hexachlorophene existed in guinea pigs with primary photosensitivity to TCSA and TBS. Photosensitivity to trichlorocarbanilide (TCC) was not produced in guinea pigs.

The guinea pig may represent a suitable animal species to study contact and photosensitivity of these and related compounds. After exposure to the photosensitizing agents has been discontinued, humans may still be sensitive to sunlight, a fact that needs further study. To a certain extent this could be due to a persistence of the chemical compound on or in the skin.

Dichlorophene used as an antimicrobial preservative may also cause contact dermatitis. Cross-sensitivity to hexachlorophene was not demonstrated.[180]

Chick Edema

Simpson et al.[181] and Sanger et al.[182] described a new highly fatal disease in chickens. The first outbreak occurred in 1957 in Georgia. Large numbers of birds from approximately three weeks of age to adult laying hens were affected. Clinical signs consisted of dyspnea, reduced body weight gain, stunting, subcutaneous edema, paleness, and sudden death. In young chickens, gasping was the first noticeable sign. This was followed by a waddling, unsteady gait. Gross inspection of the birds revealed a pale heart, fluid was noticed in the pericardial sac, and the livers were pale, mottled, and had an irregular granular surface. In advanced stages of the disease the chickens had large distended abdomens that were filled with fluid. Ecchymotic hemorrhages were present in the skin beneath the wings, on the legs, and over the Q bone in a few chickens. The kidneys were pale and swollen. Following the first outbreak, no additional cases were reported until late summer and early fall of 1957, when extensive losses of chickens and turkeys occurred throughout the southeastern part of the U.S. Suspicion soon developed that losses were caused by the consumption of rations containing certain lots of animal fat.[181] The animal fats contained tallows and greases obtained as by-products from hides preserved with chlorophenols prior to leather manufacture.[51]

This material was excluded from commercial poultry rations and no additional cases occurred where animal fats were responsible. Simpson et al.[181] came to the conclusion that the edema in the chickens was the result of vascular changes that seemed to be the primary lesion. The vascular lesion, or endotheliosis, consisted of proliferation and hypertrophy of the endothelial cells of arterioles and small arteries in most tissues. Pronounced proliferative changes in endothelium of the glomerular capillaries were observed, but no significant changes were noticed in the tubular epithelium of the kidney. Necrosis of the parenchymal cells of the liver was also noted.

In some reports it was pointed out that exposure to toxic fat resulted in a decrease of the total serum protein and a shift on the albumin to globulin ratio with a decrease in the albumin fraction. This could, of course, lead to edema formation, particularly when combined with malfunctioning glomerular capillaries. It has also been suggested that increased permeability of the cardiovascular bed contributed to the edema forma-

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tion.[180,181] In other studies the serum protein concentration did not differ from that of the controls[184,185] and increased permeability of the cardiovascular bed was advanced as the sole cause of the fluid accumulation. In addition, pulmonary edema was observed in diseased chickens and perivascular lymphocytic infiltration as well as edema of the cardiac muscle with interstitial lymphocytic infiltration has been noted.[186] Electron microscopic examination of the cardiac muscle revealed degeneration and loss of mitochondria.[184] It is possible that the heart may play a primary role in the development of chick edema, but a hepatorenal syndrome could be the underlying cause as well. Studies that investigate the development of the lesion more closely are needed to elucidate these problems. The type of diet also influenced the development of the disease. It could be brought on more rapidly with a semipurified diet than with a natural grain ration; sodium chloride in the diet was a prerequisite for the development of the syndrome.[187]

If low rations of the toxic fat were fed to chickens they did not develop chick edema but reproduction and hatchability were drastically reduced.

Toxic fat was not the only product that caused the chick edema syndrome. Chlorinated biphenyls caused the disease in chickens[188] and in Bengalese finches.[26] A mixture of pentachloronaphthalene and hexachloronaphthalene fed to chickens also resulted in chick edema disease.[189] Cantrell et al.[190] announced in 1967 that one of the toxic compounds capable of producing chick edema found in toxic fat was 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin. Then Higgenbotham et al.[52] showed that the chick edema factor was produced by two principal compounds isolated from toxic fats: 2,3,7-trichlorodibenzo-p-dioxin, and 2,3,7,8-tetrachlorodibenzo-p-dioxin. They also suggested fats and fatty acids that contain commercial chlorophenols as possible sources of contamination. When crude fats and tallows are heated to produce fatty acids, chlorophenol residues may be converted to a chick edema factor. Whether these last three compounds mentioned are the only ones that produce chick edema disease is questionable unless the chlorinated naphthalenes as well as chlorinated biphenyls that produced chick edema were also contaminated with them. Another outbreak of chick edema disease occurred in 1969.[51] In this incident millions of birds in North Carolina were involved. The outbreak was traced to a vegetable oil refinery where feed fat became contaminated with "chlorophenols." The company formulated the chlorophenols as antimicrobial water treatment products. An underground pipeline from the "pesticide" plant led to traps used to collect by-product fatty acids held as feed fats.

Flick et al.[185] have recently shown that pyrolytic products of 2,3,4,6-tetrachlorophenol were toxic to chicks and produced chick edema if 50 ppb were fed for 21 days. The product fed consisted of 53% hexa-, 45% hepta-, and 25% octachloro-p-dibenzodioxin. The pyrolytic product of pentachlorophenol consisted of a mixture of 5% hexa-, 62% hepta-, and 33% octachlorodibenzo-p-dioxin that produced mild edema and increased mortality when fed a diet containing 1 ppm (200 μg total ingestion) of the material for 21 days. When a mixture of tri- and tetrachlorodibenzo-p-dioxin was fed to the animals at a dietary concentration of 0.01 ppm (a calculated intake level of 1.9 μg), the birds developed edema and 83% of them died. Further studies with pure compounds are necessary to confirm these findings.

The effect of PCBs on the chicken was again demonstrated when PCBs that were used in the U.S. as heat transfer fluids in food processing plants contaminated large quantities of fish meal that was processed for animal feed in a plant in Wilmington, North Carolina.[191] From about April 1971 until July 1971, PCBs leaked into the fish meal and 16 thousand tons of contaminated fish meal were distributed to more than 60 companies in 10 states. One of the purchasers of the fish meal, The Holly Farms, the nation's largest poultry producer, had to slaughter 77,000 fowls after discovering contamination of the chickens with PCBs. Some of the levels found in the adipose tissue of the animals were as high as 40 ppm. The Holly Farms first discovered that the chicken meal was contaminated because the hatchability of their eggs was drastically reduced to 18% of normal.

In February and March of 1968 an outbreak of chick edema also occurred in Japan, where more than 400,000 chickens reportedly died. The disease was traced to the contamination of animal feed with Kanechlor 400, a Japanese PCB. The chick edema disease occurred simultaneously with an outbreak of polychlorinated biphenyl poisoning in people that was due to the contamination of

rice bran oil, also contaminated with Kanechlor 400 and produced by the same company.[192]

Metcalfe[193] suggested that chick edema factor could be minimized if fleshings grease from hides preserved with technical pentachlorophenols containing chlorinated dibenzodioxins were no longer used for food products. It was also pointed out that the use of glue emulsions containing chlorophenols in rendering operations is another possible source of the toxic factor. Whether elimination of these sources from animal food products will prevent chick edema epidemics remains to be seen, since polychlorinated biphenyls that may be contaminated with chlorinated dibenzofurans have also produced chick edema disease.

Yusho

During the summer of 1968, 13 cases of chloracne were observed in the western part of Japan, centering around Fukuoka prefecture. The incidence of the disease seemed to be familial and it became apparent that all patients had used the same brand of cooking oil (canned Kanemi rice oil). These findings were picked up by the newspapers and a strong demand was voiced to elucidate the cause. Therefore, a Yusho study group was established by Kyushu University.[192] The term Yusho stands for rice oil disease. After extensive investigations it was found that several illnesses occurred in a number of households that had used common cans of rice bran oil.

When a total number of 89 males and 100 females that suffered from Yusho were examined, a variety of symptoms were found in these patients. The incidence of the different symptoms that varied a great deal is given in Table 2. The epidemic outbreak was finally, after very extensive investigation, pinpointed to Kanemi oil, manufactured on February 5, 1968. Chemical analysis of this oil established that it was contaminated with 2,000 ppm of Kanechlor 400, a chlorobiphenyl that contains 48% chlorine. It was also demonstrated that most of the components of the chlorobiphenyls were stored in adipose tissue of the patients for quite a long time and passed through the placenta into the fetus of a few women who happened to be pregnant.[194]

The amount of oil necessary to produce disease was found to be at least 2.5 l. The younger the patient, the higher the morbidity rate with a smaller total amount of ingested oil. The average amount of chlorobiphenyl consumed by individual patients was calculated to be a total of 2 g.[195] (Various reports written on this subject give different accounts of what actually was consumed and the amount can at best only be estimated.)

When the rice bran oil was heated under reduced pressure, Kanechlor leaked from the old pipes in which holes were subsequently discovered.

The Yusho outbreak spread not only over Fukuoka, Japan, but also over 20 other prefectures in the western part of Japan. A total of 1,057 poisoning cases resulted according to the latest tabulation (August 1971, Ministry of Welfare).[196] The most common initial symptom experienced by 136 patients with Yusho was increased eye discharge and swelling of the upper eyelids (38.3%) followed by acneform eruption, follicular accentuation (33.1%), and pigmentation of the skin (9.6%). Itching of the skin and "stiffening" of the soles of the feet and the palms of the hands were also observed. Pigmented mucus membranes were noticed in many of the patients; a small percentage of them developed jaundice, many complained of a feeling of weakness and headaches; occasionally vomiting, diarrhea, and fever also occurred. Various neurological symptoms manifested themselves such as transient visual disturbances, numbness and spasms in the limbs, and hearing difficulties. Most patients showed acnelike skin eruptions that resembled the chloracne observed after occupational exposure to chlorinated naphthalenes or chlorinated biphenyls. When the age and sex specific incidence rates were analyzed it was found that a significant sex difference was not present, but a lower risk for both males and females in the age groups over 60 years was noted. The proportion of severe cases among those age 13 to 29 was significantly larger than that of other age groups.

During the Yusho epidemic, 11 women with Yusho and 2 wives whose husbands had Yusho, but who themselves did not show the disease, delivered 10 liveborn and 2 stillborn babies. Nine of these babies had unusually greyish, dark brown stained skin, and dark pigmentation of the gingiva and nails was noted in five of them. Most infants also had heavy eye discharge. Histological examination of a stillborn fetus showed a marked hyperkeratosis and atrophy of the epidermis and cystic dilation of hair follicles, especially of those of the head. A marked increase of melanin pigment in the basal cells of the epidermis was also noted. Most fetuses that were born were smaller

TABLE 2

Symptoms (complaints) of Patients (89 males, 100 Females, as of October 31, 1968)*

Symptoms	Males %	Females %
Blackening of nails	83.1	75.0
Black spots in all pores	64.0	56.0
Excessive sweating in palms	50.6	55.0
Acnelike skin eruptions	87.6	82.0
Red spots on limbs	20.2	16.0
Itching	42.7	52.0
Change in skin color	75.3	72.0
Swelling of hands and feet	20.2	41.0
Hardening of backs and hands	24.7	29.0
Pigmentation of mucous membranes	56.2	47.0
Sebum (gum secretion in eyes)	88.8	83.0
Hyperemia of mucous membranes in eyes	70.8	71.0
Temporary failing of eyesight	56.2	55.0
Jaundice	11.2	11.0
Swelling of upper eyelids	71.9	74.0
Sense of weakness	58.4	52.0
Numbness of hands and feet	32.6	39.0
Fever	16.9	19.0
Hearing difficulty	18.0	19.0
Spasms of hands and feet	7.9	8.0
Headaches	30.3	39.0
Vomiting	23.6	28.0
Diarrhea	19.1	17.0

*Reprinted with permission from Kuratsune et al.[193]

than the national standards and four of them were small for gestational age babies. As they grew older their skin gradually became lighter over a period of three months.

Okumura and Katsuki[197] studied a group of 24 patients whose main complaint had been acneform skin eruptions and who had been diagnosed as definitely having Yusho. Eighteen of these were adults and six were children. Irrespective of the seriousness of the disease in all patients, the initial symptoms were eye discharge associated with edema of the eyelids, defects in vision, generalized fatigue, anorexia followed by comedo and acneform eruptions of the face, and pigmentation and flattening of the nails. The principal subjective symptoms were fatigue, numbness of the lower limbs, and intolerance for *sake*,* which is a Japanese alcoholic beverage made from fermented rice. Three of twelve females had an irregular menstrual cycle. Seven of nine patients with serious disease showed a low grade fever. Most laboratory tests conducted on these patients were normal, including serum enzyme values. A slight elevation in alkaline phosphatase was observed in over half of the seriously ill groups. An increase in the α_2 globulin fraction of the serum protein was noted with a concomitant reduction of the albumin fraction, but the total serum proteins were within the normal range. These same 24 patients had abnormally elevated serum triglycerides ranging from 200 to 600 mg %. In 12 of 24 cases, while the total serum cholesterol remained unchanged, phospholipids tended to be somewhat lower.[198] Preliminary animal experiments in rabbits conducted by the same authors showed that lipemia was consistently induced within several days. The levels returned to their previously normal values several weeks after exposure had been discontinued.

The serum concentration of triglyceride is usually elevated in humans in glycogen storage disease (Von Gierke's disease). An increase in plasma triglyceride concentration is also observed in *essential hyperglyceridemia*, an apparent con-

genital anomaly. Essential hyperglyceridemia has been observed in siblings of a number of families, but the mode of transmission is not known. The course of this disease is generally benign and is consistent with a normal life span.[199] Observation of the familial cases shows that the mere elevation of triglyceride does not in itself increase the risk of arteriosclerosis.

Another interesting fact in this poisoning epidemic was that out of 23 cases with obvious Yusho, 10 showed symptoms or signs of a sensory neuropathy that included numbness, pain and hypoesthesia, and, in addition, 1 case also showed areflexia.[200,201] This effect on the sensory nerves would not be immediately obvious in animal experiments and probably should be investigated further.

Samples of sputa and adipose tissue collected from patients who suffered from Yusho contained chlorinated biphenyls on chemical analysis.[194] The concentration in adipose tissue was much higher than in sputa. Recently Kuratsune et al.[196] reported on the present status of the patients who suffered from Yusho. Many of the patients were still suffering from the illness. When the findings made in 150 patients in 1970 were compared with those observed in 1969, it was found that half of the patients had improved clinically, while the remaining half showed no such improvement and more than 10% of the patients had become worse.

Immunosuppression

Several reports have indicated that PCBs and possibly some of the chlorinated dibenzodioxins and furans may alter the immune response in animals. Atrophy of the thymus and reduced total white count were observed in rabbits following the dermal exposure to PCB fractions containing tetra- and pentachlorodibenzofuran.[96] In fish, exposure to Aroclor 1254 for 14 to 45 days led to emaciation and funguslike skin lesions.[93] However, no attempt was made to isolate organisms from these lesions. When 10-day-old mallard ducklings were fed diets containing PCBs (Aroclor 1254) and were subsequently injected interperitoneally with a duck hepatitis virus, significantly more ducklings died than when they received the virus alone.[202] In guinea pigs, sublethal dosage levels of 2,3,7,8-tetrachlorodibenzodioxin produced severe atrophy of the thymus with destruction of lymphocytes, lymphoid depletion in spleen and lymph nodes, as well as extensive hemorrhages into various organs including the adrenals. Reduced thymus and spleen weights were also observed in rats that received a single dose of 25 μg/kg TCDD and in mice the cell mediated immunity was found to be suppressed.[203] The various findings listed here do not conclusively prove that the immune response is specifically altered by these compounds as we encounter it following radiation exposure or therapy with alkylating agents. In most studies relatively high doses of the chemical compounds were given, resulting in general toxicity accompanied by weight loss. Weight loss in itself may lead to atrophy of the spleen. In humans maintained on a low caloric intake with negative nitrogen balance, a general reduction of lymphoid tissue results which is not specifically related to the immune response and vitamin A deficiency is also accompanied by a reduction in lymphatic tissue. In this respect it is also of interest that when monkeys were fed "toxic fat" containing polycyclic polychlorinated hydrocarbons. The germinal centers in the lymph nodes and spleen as well as islands of hematopoietic cells in bone marrow were extremely sparse.[160] This is more extensively discussed in connection with chick edema. The fact that a combination of duck hepatitis virus and PCBs is more toxic to 10-day-old ducklings than the virus alone suggests a reduced immune response but could also merely represent an additive damaging effect on the liver, made more susceptible to infection by the toxic effects of PCBs. So far no convincing evidence of "wasting disease" as a result of exposure to chlorinated polycyclic compounds has been reported in rodents or any other species in the literature.

Atrophy of the thymus in newborn rodents reduces the capacity of these animals to produce serum antibodies, resulting in wasting disease, also referred to as runt disease or homologous disease. Such animals are then incapable of rejecting foreign skin grafts,[204] which probably represents the most convincing evidence for homologous disease. A shift in the immune response could influence the development and growth of cancer and alter the defense mechanism in infections. In order to prove that PCBs as well as chlorinated dibenzodioxins and furans do indeed influence the immune response, the effect of these substances on newborn animals of several species should be tested with adequate parameters such as skin

grafting and the measuring of the development of antibodies. Whether the other chlorinated polycylic compounds in this article also affect the immune response has not been suggested or reported.

Biochemical Effects and Chemical Toxicology

Porphyria

Porphyria cutanea tarda in humans is an acquired defect in hepatic porphyrin metabolism, characterized by uroporphorinuria, photosensitivity as manifested by blisters, and mechanical fragility of the skin. The hepatic porphyria (Porphyria cutania tarda) which is responsible for the increase in porphyrins and the skin photosensitivity can be produced experimentally by a number of drugs; all of these have the ability to stimulate the activity of the initial enzyme in the heme synthesis δ-aminolevulinic acid synthetase (ALA). In addition to this acquired porphyria, an acute intermittent porphyria erthropoietica and an acute intermittent porphyria hepatica, which are both hereditary, exist together with a number of subgroups which will not be considered here.

In 1964, Bleiberg et al.[2] reported studies of 29 workers in a 2,4-D (2,4-dichlorophenoxyacetic acid) and a 2,4,5-T (trichlorophenoxyacetic acid) factory. Many of the workers had chloracne and 11 had abnormal excretion of urinary uroporphyrins. Many of the workers with uroporphyrinuria had hirsutism, hyperpigmentation, and increased skin fragility. Liver dysfunction was noted in two hospitalized patients. Poland et al.[1] reinvestigated the workers of the same plant studied by Bleiberg et al.[2] They studied a total of 73 male employees; chloracne was found in 80% of the workers. In this study six years after the first report, clinical porphyria was not observed and only one worker had persistent uroporphyrinuria. In the Japanese poisoning epidemic (Yusho), increased uroporphyrin excretion was not reported.

Apparently polychlorinated biphenyls, as well as tetrachlorodibenzodioxins, produce hepatic porphyria in animals. Experimental hepatic porphyria was observed in Sherman strain rats when the rats were exposed to Aroclor 1254 in the diet. The porphyria resembled hexachlorobenzene poisoning and human porphyria cutanea tarda.[205] When female rats were fed 100 ppm Aroclor 1254 in the diet (approximately 7.9 mg/kg/day) they became porphyric after a delay of about 2 to 4 months. At this time the urinary porphyrin excretion increased rapidly. Uroporphyrin excretion was elevated to a maximum of 550-fold, prophobilinogen 27-fold, and δ-aminolevulinic acid 18-fold. The major porphyrins found in urine from Aroclor treated rats were 8-carboxyporphyrin (73%) and 7-carboxyporphyrin (16%). Substantial increases in amounts of 5 and 6 carboxcylic porphyrins were also observed. A 10-fold increase in the excretion of coproporphyrins was observed, but the increase in uroporphyrin excretion was 1,400-fold. In control rat urines, coproporphyrin (81%) represented the major porphyrin fraction. A good correlation between urinary porphyrins and liver porphyrins was also observed. Only a trace of porphyrin was found in liver samples in control rats.

When feeding rats 100 ppm Aroclor 1254, δ-aminolevulinic acid synthetase was not increased in the first month of exposure. After this time δ-aminolevulinic acid synthetase was elevated 3- to 6-fold in porphyric rats but was normal in nonporphyric Aroclor treated rats. Cytochrome P-450 and microsomal heme were also increased throughout the study but the increase was maximal in one week despite the absence of an increase of the rate limiting enzyme in heme synthesis δ-aminolevulinic acid synthetase. When a large single dose of Aroclor 1254 (500 mg/kg) was given, a 4-fold induction of δ-aminolevulinic acid synthetase could be demonstrated in the liver 5 hr after dosing the animals. These results suggested that δ-aminolevulinic acid synthetase induction occurred when porphyria had developed if lower levels of Aroclor 1254 were fed over a period of time, but when high doses of Aroclor were given it was induced immediately.

Vos and Koeman[95] reported that chemical porphyria was produced by the two European PCB products, Phenoclor DP6 and Clophen A60, as well as the American product, Aroclor 1260. The porphyrogenic action was observed in chickens, quail, and rats. It has not been established so far whether only certain isomers of the polychlorinated biphenyls or contamination with a chlorinated dibenzofuran is responsible for the production of hepatic porphyria cutanea tarda.

Poland and Glover[206] used fertile chicken eggs at a developmental stage of 15 to 20 days and injected them with 25 μl of the *halogenated dibenzo-p-dioxins*. They found that 2,3,7,8-tetrachlorodibenzo-p-dioxin produced a dose-related

increase in ALA synthetase activity. The lowest dose tested, 4.66×10^{-12} mol/egg (1.5 ng) doubled the enzyme activity. These results were statistically significant.

Studies with other halogenated dibenzo-p-dioxins gave the following results: the 2,3,7-trichloro and 2,3,6-tribromo isomers are potent inducers of ALA synthetase while 2,3-dichloro and 2,7-dichloro, 2,8-dichloro, 1,3,6,8-tetrachloro, and 1,2,3,4-tetrachloro isomers all failed to induce ALA synthetase at doses up to 2.5 mg/egg. Of the limited number of halogenated dibenzo-p-dioxins that have been tested for fetal toxicity and the ability to produce chloracne, those that are toxic at low doses also induce ALA synthetase. Whether some of the polyneurotic symptoms reported in Yusho and also in severe exposure to technical 2,4,5-T or technical trichlorophenol that were heavily contaminated with tetrachlorodibenzodioxin are caused by an effect on the porphyrin metabolism merits investigation since neurotoxic effects have been reported in acute intermittent porphyria.[207]

The dioxins that induce ALA synthetase in the liver of the chick embryo may also induce it in the mammalian system. Woods[208] was not able to induce δ-aminolevulinic acid synthetase (ALA synthetase) in the liver of male rats up to 30 days after oral doses of 2,3,7,8-tetrachlorodibenzo-dioxin. Guinea pigs and mice also did not show an increase of ALA synthetase following the administration of 2,3,7,8-tetrachlorodibenzodioxin. However, it was possible to demonstate an increase in ALA synthetase and uroporphyrins in the liver in C_{57} black male mice given 25 μg/kg/week for 4 weeks.[209] It is possible that ALA synthetase induction varies in time with age and sex and may show species variation. Induction in mammals may require longer dosing, since, for instance, porphyria and induction of ALA synthetase with moderate doses of Aroclor 1254 did not develop until 2 to 4 months after dosing was started.[205] Female animals and the embryo may be more susceptible than adult male animals.

Effect on Other Enzymes and on Vitamin A

A number of investigators have shown that the *polychlorinated biphenyls* induce liver microsomal enzymes.[210,211] Norback and Allen[211] correlated an increase of hepatic microsomal enzyme activity with smooth endoplasmic reticulum proliferation. Litterst et al.[212] studied the effect of four PCB mixtures, namely, Aroclor 1242, 1248, 1254, and 1260. The animals were fed the material for 4 weeks and dietary levels employed were 500, 50, 5 and 0.5 ppm. At higher dietary levels an increase in liver weights was observed. Elevation of triglycerides in the liver was seen at 500 ppm with a maximum effect occurring at approximately 50% chlorine content. An increase in cytochrome P-450 was observed at the 50 and 500 ppm dietary levels of all PCB mixtures. An induction of the pentobarbital hydroxylation and an increase in methylase activity were observed at the higher dietary levels. Induction of pentobarbital hydroxylation increased with increasing chlorine content of the PCBs. Nitroreductase activity was markedly stimulated with increasing doses of PCB and the induction was seen at levels as low as 0.5 ppm for all mixtures. It is difficult to evaluate the enzyme induction at these low dietary levels since the control animal food can be contaminated at times with PCBs.

Two types of inducers of drug-metabolizing enzymes of the liver have been reported; one group to which phenobarbital belongs resulted in increased P-450 content of the liver and induction of a variety of pathways including benzpyrene hydroxylase and demethylation of ethylmorphine. The polycylic hydrocarbons such as 3,4-benzpyrene belong to the second group, which resulted in the formation of an abnormal cytochrome, P-448, and increased benzpyrene hydroxylation, but not ethylmorphine demethylation. Alvares et al.[213] reported that PCB treatment produced an increase in cytochrome P-448, an increase in benzpyrene hydroxylation, and N-demethylation. Since the PCBs represent mixtures, it is possible that the type of induction may depend upon the isomers present. The various effects of PCBs on liver microsomal enzymes can be blocked by the prior administration of actinomycin D. When the effect of a number of PCBs on the beef heart mitochrondrial enzyme system was studied, it was found that they inhibited NADH-oxidase, succinoxidase, and cytochrome oxidase activity. It has not been established whether these same effects will occur in vivo. Benthe et al.[214] induced liver microsomal enzymes by intraperitoneal injection of a single dose of Aroclor 1248 or 1232.

Most of the microsomal enzymes have been studied in the rat, but they are affected in other species as well. Allen et al.[215] reported liver

hypertrophy after feeding polychlorinated biphenyls (Aroclor 1248) or a polychlorinated triphenyl (Aroclor 5460) for three months to primates. The increase in liver size was attributed to the increase in smooth endoplasmic reticulum and induction of microsomal enzymes was observed. Proof of the induction of microsomal enzymes in birds is the finding made by Lincer and Peakall[216] that livers obtained from American kestrels exposed to either Aroclor 1254 or Aroclor 1262 showed an increase in the in vitro breakdown of oestradiol to a more polar metabolite. This effect on sex hormones is also emphasized by Platonow et al.[217] Aroclor 1254 given to Yorkshire boars reduced the urinary excretion of gonadal steroids.

Both the *polychlorinated biphenyls* as well as the *chlorinated naphthalenes* affect the vitamin A content of the liver. This was particularly studied with the chlorinated naphthalenes since hyperkeratosis and other abnormal keratin formation of the skin is often accompanied by vitamin A deficiency. Low vitamin A plasma levels were observed in cattle with X-disease and young swine.[155,162] A decrease in vitamin A storage in the liver of Japanese quail and rats that were fed Aroclor 1242 was also demonstrated.[218] Since vitamin A deficiency affects reproduction, this aspect should be further investigated to determine whether the effect of PCBs on reproduction is secondary to vitamin A deficiency.[219]

Ecobichon and Johnstone[220] studied the effect of various PCB isomers on the hepatic enzyme system. Biphenyl itself did not cause induction of hepatic drug metabolizing enzymes and neither did monochlorobiphenyl (4-Cl). Substitution of chloro groups at the three and four positions of the di- and tetrachlorobiphenyls caused induction of microsomal monooxygenases. Induction of microsomal monooxygenases occurred also when hexa- and octachlorobiphenyls were given to the rats. Nitroreductase and carboxylesterase activities were not affected while all compounds produced a marked induction of the sulfobromophthalein conjugating system. *Terphenyls* as well as chlorinated terphenyls increased the pentobarbital metabolism in the rat with corresponding increase in smooth endoplasmic reticulum which suggested microsomal enzyme induction.[221,222]

Lucier et al.[223] reported that a single oral dose of 5 or 25 μg/kg body weight of *tetrachlorodibenzodioxin* induced cytochrome P-450 significantly in male rats for a period of up to 28 days. Cytochrome b-5 content was also increased. Hydroxylation of aniline was induced and aminopyrine demethylation rates were decreased. Microsomal proline was increased by approximately 15%. The induction of UDP glucuronyltransferase was most striking. Oxidative phosphorylation rates in rat liver mitochondria using succinate as a substrate were unchanged. The enzyme induction was observed as early as one day – the peak induction was reached between three and ten days after treatment. The enzyme induction was accompanied by an increase in smooth endoplasmic reticulum and a mild increase in rough endoplasmic reticulum.[224] These changes were first observed three days after the injection of the tetrachlorodibenzodioxin. Thus, enlargement of the liver during exposure to various chlorinated compounds is accompanied by an increase in smooth endoplasmic reticulum.

Furthermore, if "inclusions" or "hyaline bodies" are seen within the cytoplasm with the light microscope, concentrically arranged membranes that often surround lipid vacuoles can be seen with the electron microscope (Figure 6).

Hexachlorophene, on the other hand, does not increase the size of the liver.[118] The livers show no ultrastructural changes and probably microsomal enzymes are not induced. Whether other compounds listed in Figure 2 affect liver microsomal enzymes is not known. Hexachlorophene has an effect on a variety of other enzyme systems than those mentioned thus far. Cammer and Moore[225] and Caldwell et al.[226] have shown that hexachlorophene uncoupled oxidative phorphorylation, increased adenosine triphosphatase activity, but did not affect the activities of electron transport enzymes when compared to controls. Gould et al.[227] demonstrated that hexachlorophene inhibited succinoxidase activity of rat heart, liver, and kidney preparations. According to unpublished material cited by Gluck,[228] hexachlorophene is conjugated to the glucuronide conjugate which is excreted in bile. The proportion of conjugated hexachlorophene to unconjugated hexachlorophene in blood may be more significant in determining the toxicity of hexachlorophene than the hexachlorophene blood levels as they are presently determined.

Methemoglobinemia

In the early 1960s outbreaks of methemoglob-

inemias were reported from hospital nurseries.[229,230] One outbreak involved a total of 18 infants with a level of methemoglobin from 4.9 to 30% of the total hemoglobin. An epidemiological investigation traced the outbreak to *3,4,4'-trichlorocarbanilide (TCC)* that was used as a 2% solution in a rinse in the laundering process, after which the laundry was neutralized with an acid and then dried in an autoclave. Twelve of the eighteen cases in this report were prematures who were probably longer exposed to the offending chemical than term infants since their hospital stay is usually longer. It is also possible that chemicals penetrate more easily through the skin of prematures.

In another outbreak, methemoglobinemia occurred among a group of custodial patients in an institution. These patients were given soap suds enemas with boiled soap that contained 2% TCC.[231]

It is possible that by heating TCC a breakdown product occurred which produced the methemoglobinemia. P-chloroaniline and 3,4-dichloroaniline have been suggested as breakdown products. None of the other compounds discussed in this article have thus far been shown to produce methemoglobinemia.

Storage, Distribution, Metabolism, and Excretion

As we progress further in our study on the distribution, metabolism, excretion, and absorption of compounds such as chlorinated biphenyls, chlorinated terphenyls, the chlorinated dibenzodioxins, and chlorinated naphthalenes, we will probably find that since they are lipid soluble they are stored in quite the same way as the more studied persistent pesticides such as DDT and dieldrin. From the evidence presently available on the chlorinated biphenyls it can be assumed that the turnover of these compounds in the body, particularly of those that have a high chlorine content, may be slower than some of the persistent pesticides. The turnover rate will vary with the degree of chlorination. Hexachlorophene, on the other hand, is fairly rapidly excreted and presently no information is available on bithionol and related compounds that have a germicidal effect. All of these compounds are not very well absorbed by the gastrointestinal tract or the skin.

Analysis of various tissues of rats given a single dose of the PCB Aroclor 1254[232] showed the highest concentration of PCBs in adipose tissue and the least in plasma. Brain, liver, and kidney had levels of the same order of magnitude but were lower. If, for instance, 1,600 mg/kg body weight of Aroclor 1254 were given by stomach tube, 24 hr later the mean concentration in adipose tissue was 1,146 ppm. The concentrations observed in individual animals varied greatly. Similar levels were found in adipose tissue when 3,200 mg/kg of Aroclor 1260 were given by stomach tube to adult female rats. The levels of Aroclor 1254 found in the brain showed a mean of 138 ppm and the values observed for Aroclor 1260 were 145 ppm. All of the values were calculated on a wet weight basis. The same concentrations in ppm were found with both compounds even though only half of the amount of Aroclor 1254 was given. This is probably due to the fact that only a certain amount of Aroclor is absorbed at a given time.

When 100 ppm of Aroclor 1254 was fed to male weanling rats for 58 days the mean concentration of Aroclor derived materials found in the feces varied roughly between 11 and 35 ppm. After the animals had been removed from the experimental diet for a week, the excretion in the feces was 8 ppm and after 16 days' recovery an average of 2.5 ppm was found in the feces. PCBs at the concentration of about 2 ppm were still observed in the feces 24 days after the animals were removed from the experimental diets. During the entire study the amount of PCB derived material recovered from the urine remained below 1 ppm. While PCBs were fed to the rats, the gas chromatogram obtained from the feces was superimposable on the one obtained from the standard Aroclor 1254 that was fed to the rats. Once the animals were no longer fed Aroclor 1254 the number of peaks observed in the gas chromatograms that resulted from the analysis of the fecal material decreased. Fewer peaks were also observed in the gas chromatograms obtained for the tissues and from urine. During the 58-day feeding period, a gradual increase in the concentration of the PCB derivatives was observed in tissues. The highest concentration was found in adipose tissue; after 58 days it reached mean values of about 500 ppm. The concentration in the liver only amounted to a mean of 12 ppm at that time. Following a recovery period of 71 days when the animals were fed plain chow, the concentration of the PCB derived material in adipose tissue had not decreased appreciably and

still showed values in the neighborhood of about 400 ppm and 4.7 ppm was found in the liver. The concentration in the brain never reached very high proportions; it ranged from about 2 to 4 ppm. Following a 71-day recovery period the amount in the brain was still about 2 ppm. When Aroclor 1254 was fed for 240 days at the dietary level of 100 ppm, the concentration of the PCB derived material observed in adipose tissue had reached values of about 1,000 ppm. If, on the other hand, 100 ppm of DDT is fed to male rats for 2 years, the concentration in adipose tissue is about 95 ppm.[233] In a separate study where 500 ppm of Aroclor 1254 was fed to male rats for 6 months and the exposure to PCB containing diets was subsequently discontinued for an additional 10 months, the concentration of the PCBs ranged from 924 to 1,688 ppm in adipose tissue with an arithmetic mean of 1,192 ppm. The concentrations in the liver ranged from 17.3 ppm to 26.24 ppm with an arithmetic mean of 22.65 ppm.[234]

Further studies are necessary to determine whether PCBs are stored in tissues to a certain point when a steady state is reached and the concentration does not increase further. The given concentration at which this equilibrium is reached in adipose tissue would vary with the amount fed to the animals and the type of compound. The effect of starvation or illness when the amount of adipose tissue in the mammalian body is suddenly reduced also needs to be investigated further. Would this lead to increased excretion of the material or to redistribution or both? Dahlgren et al.[235] who determined PCB tissue levels in pheasants, found that a concentration of 300 to 400 ppm of the PCB derived material was usually found in the brains of birds that had died, while live birds that were fed the same amount of PCBs had much lower concentrations in their brain tissue.

Studies in bobwhite quail showed that relative peak heights and chromatograms for the tissue extracts were similar to the Aroclor standard after partitioning with acetonitrile-hexane when the birds were given a single capsule of Aroclor and their carcasses were analyzed soon after exposure. This suggests absorption of all components of Aroclor. The gastrointestinal tract, liver, skin, wings, and feet had been removed prior to analysis. When bobwhite quail were fed Aroclor 1254 for 14 days and then allowed to recover, the gas chromatograms showed a gradual reduction in the number of peaks and after 42 days on a control diet only 4 major PCB components remain, which suggested selective excretion and/or metabolism of some of the components of the Aroclor tested.[236] In a reproduction study, Heath et al.[99] analyzed 2 second-year eggs of mallards fed 25 ppm Aroclor 1254 in their diet for 2 years and found 56 and 33 ppm PCB by wet weight. The gas chromatogram showed a pronounced diminution of the early peaks when it was compared to the standard, suggesting again a selective reduction of the components with lower chlorine concentrations. This is also evident in environmental samples. Lower organisms such as mussels and fish contained a higher concentration of PCBs with lower chlorination than birds.[15]

In mammals, excretion of PCBs in milk and transplacental passage is also observed. Feeding cows 200 mg/day of Aroclor 1254 for 60 days[28] resulted in higher PCB levels in milk fat than in body fat. At 30 days' exposure, 45.6 ppm was found in milk fat and 21.9 ppm in body fat; at 60 days' exposure, 66.7 ppm was found in milk fat and 44 ppm in body fat. However, once exposure to PCBs was discontinued, the concentration in milk fat sank below that of body fat.

Transplacental passage of PCBs also occurred.[237,238] Sherman strain rats were given Aroclor 1254 in peanut oil by stomach tube during days 7 to 15 of gestation. A mean of 20.60 ppm PCB derived material was found in their milk when 10 mg/kg/day was given to the dams during pregnancy, while those given 50 mg/kg/day excreted a mean of 66.34 ppm in their milk on a wet weight basis. Fetuses taken by Caesarean section on day 20 of pregnancy contained a mean of 0.63 ppm and 1.38 ppm of PCBs, respectively. With a fivefold difference in dosage, the concentration in fetuses showed a twofold difference and in milk a threefold difference.[238]

The hyperpigmented babies observed in the Yusho incidents in Japan represent additional circumstantial evidence of transplacental passage of PCBs.

Polychlorinated biphenyls have also been detected in human adipose tissue. Biros et al.[239] analyzed two human adipose tissue samples which contained 200 and 600 ppm PCBs ranging from pentachlorobiphenyl to decachlorobiphenyl and Price and Welch[240] found 100 ppm PCBs in another human adipose tissue sample. According

to Price and Welch,[240] 41 to 45% of the general population in the U.S. contain 1 ppm or more PCBs in their adipose tissue. Among PCB sources they discussed the food chain and house dust which, from residences in southwestern Michigan, contained up to 180 ppm PCBs. Polychlorinated biphenyls were found in 31% of 637 samples of human adipose tissue that were collected from the general population of the U.S., as part of the Human Monitoring Survey. Of the samples analyzed, 5% contained more than 2 ppm.[241] PCBs have also been found in human adipose tissues of the general population of Scandinavia[242] and Japan,[244,245] and in human milk and human adipose tissue of the general population of Germany.[243] In general, low concentrations of PCBs were stored in adipose tissue in a certain proportion of the general population. This is probably more prevalent in the highly industrialized countries and almost nonexistent in underdeveloped countries unless most of the food was imported from polluted areas. These low levels of PCBs are harmless, as far as we know at the moment; however, the occasional levels found in the 100 to 1,000 ppm range give rise to concern. It would be of interest to establish whether average adipose tissue levels increase with age and whether those in certain professions such as painters, printers, textile workers, workers in plastic factories, and those engaged in the manufacture of transformers and capacitors had higher PCB levels than the general population.

Reports on the absorption, storage, and metabolism of chlorinated naphthalenes, terphenyls, dichlorophene, bithionols, fenticlor, triclosan, and tetrachlorosalicylanilide are almost nonexistent in the literature. Some unpublished results on some of the compounds used as *germicides* are mentioned in the summary minutes of the Over the Counter Drug Panel on antimicrobials. Copies can be obtained by writing to Dr. Mary Bruch, Bureau of Drugs, Rockville, Md.

Approximately 9% of *triclosan*, also called Irgasan DP300 and CH 3565 (2,4,4'-trichloro-2'-hydroxydiphenyl ether), is absorbed when it is applied dermally in a soap solution. The halflife of the chemical was 20 hr when applied dermally and 10 hr when given intravenously. Humans excrete 65% in urine and 20% in feces after intravenous injection. It is excreted either as a free compound or as a glucuronide.

In dogs nearly 100% of the injected material is recovered as the glucuronide or sulfate in urine and feces over a 5-day period. It has been shown that TCC is absorbed through the skin.

Tissue levels of the *chlorinated terphenyl* Aroclor 5460 were determined in codfish.[246] In this study it was found that Aroclor 5460 was apparently poorly absorbed from the gastrointestinal tract; it was stored in all tissues that were analyzed. The highest concentration was found in the liver and following a single dose of 1 g some of it was still present in the codfish after 70 days, an indication of slow excretion. The authors pointed out that with the method used to determine the chlorinated terphenyls, it would be difficult to distinguish chlorinated biphenyls from chlorinated terphenyls. A comparison of the chromatograms of the standard with those obtained from chlorinated terphenyls extracted from tissues revealed more of the earlier peaks in material obtained from tissues and fewer of the later peaks. A variation in the intensity of the peaks was also observed. There seems to be a change in the chlorinated terphenyls within the living organism; however, part of this could also have been introduced as an artefact by the analytical methods or by differential absorption from the gastrointestinal tract.

Calves born to cows with X-disease developed hyperkeratosis and it was found that highly *chlorinated naphthalenes* were excreted in milk.[156] This suggests that chlorinated naphthalenes may well follow similar storage and excretion patterns as PCBs.

Chlorinated dibenzodioxins and *furans* are probably also stored in tissues of animals and men who are exposed to them, but again not much information is presently available. Firestone et al.[247] fed the unsaponifiable fraction isolated from "toxic fat" or 3% "toxic fat" itself to young cockerels. The "toxic fat" contained chlorodibenzodioxins with 2 to 8 chlorine atoms. When the excreta were analyzed it was found that a certain percentage of the hexachlorodibenzodioxins had been absorbed from the gastrointestinal tract, while all of the octachlorodibenzodioxins remained in the gastrointestinal tract, and only very little of the heptachlorodibenzodioxin was absorbed. Because of the presence of interfering components, the chick tissues and excreta could not be examined for the lower chlorinated dioxins. In the organs, the highest concentration was found in the liver and an appreciable amount

was also found in the bone and skin, while the other organs contained very few dioxins. Some evidence for a possible metabolism of hexa- and heptachlorodibenzodioxin by the chick was also observed. Zitko and Wildish[248] fed di-, tri-, tetra-, and octachlorodibenzofuran to fish and detected only octachlorodibenzofuran in muscle and gut of dead fish. One problem with metabolism, storage, and distribution studies of chlorinated dibenzodioxins and furans is that not all of the compounds are readily available and, if they are available, any long-term feeding studies in large animals are expensive. The very toxic dioxin compounds can be given only at such low levels that they escape detection easily. In our laboratory we detected a chlorinated dibenzofuran in the urine of rats fed Aroclor 1254. We found this same chlorinated dibenzofuran in the Aroclor itself[40] as a contaminant.

Williams et al.[249] fed rats a total of 22.7 and 120.7 μg of octachlorodibenzodioxin. They recovered a great deal of the compound in the feces. Very small amounts were recovered in the liver at both dosage levels and in adipose tissue at the higher dosage level. Further studies are needed to establish the absorption, excretion, and storage of these compounds, and their persistence. Studies with labeled material may eventually give us some information on their metabolism. Piper et al.[250] gave a single oral dose of C^{14} labeled 2,3,7,8-tetrachlorodibenzo-p-dioxin to rats and found that about half of the radioactive material was recovered within 21 days in the feces and only 13% in urine and 3% in expired air. Most of the remaining radioactive material was found in the liver.

In another study[251] 100 μg of Cl^{36}-octachlorodibenzodioxin was given daily to rats by stomach tube for 21 days. Less than 0.3% of the material was absorbed. Subsequently the rats received a control diet for 7 weeks and still showed 20% of the absorbed material found at 21 days in their tissues.

The in vivo metabolites of the various compounds under discussion have in most instances not been identified. Block and Cornish[252] studied the urinary excretion of biphenyl and 4-chlorobiphenyl in rabbits. Biphenylglucosiduronic acid and 4-hydroxybiphenyl were isolated from urine of rabbits fed biphenyls. The rabbits fed the 4-chlorobiphenyl excreted 4-(p-chlorophenyl)-phenol and 4-chlorobiphenyl glucosiduronide. Twice as much 4-chlorobiphenyl as biphenyl was excreted as the glucosiduronic acid derivative. It is possible that other low chlorinated biphenyls and naphthalenes are excreted in urine in a similar manner; whether they would be detected by present gas chromatographic methods would have to be checked by combining the identification of radio labeled material with gas chromatography.

Hutzinger et al.[253] found evidence that some chlorinated biphenyls are hydroxylated by some species. Apparently trout is not able to hydroxylate 4-chlorobiphenyl, 4,4′-dichlorobiphenyl, and 2,2′,5,5′-tetrachlorobiphenyl, while the rat and the pigeon both formed monohydroxychlorobiphenyl from 4-chlorobiphenyl. In addition, the rat also produced dihydrochlorobiphenyl. Large quantities of unchanged material were excreted by the rat when 4,4′-dichlorobiphenyl and 2,2′,5,5′-tetrachlorobiphenyl were given to them and only a monohydroxyderivative was identified in their urine. Pigeons followed a similar pattern but no dihydrochlorobiphenyl was observed in the bird excreta fed 4-chlorobiphenyl. No hydroxy metabolites could be identified in the excreta of the three species when they were given 2,2′,4,4′5,5′-hexachlorobiphenyl. No evidence of reductive dechlorination was observed in any species.

Only very few studies have been conducted with radiolabeled *hexachlorophene* to study its absorption, distribution, and metabolism. Wit and Van Genderen[254] gave rats and rabbits hexachlorophene-C^{14} (2,2′-methylene-C^{14} bis(3,4,6-trichlorophenol). Complete recovery of radioactivity in excreta after a single dose of 50 or 15 mg/kg of radioactive hexachlorophene was observed in rabbits within 5 days. Roughly one third was excreted in urine and feces, respectively, as unchanged hexachlorophene and the remainder was excreted in the feces as unidentified metabolites. The metabolites could not be extracted from the feces with ethanol. The biological halflife calculated from the experimental results was found to be approximately 17.8 hr. In rats, about two thirds of the radioactivity was recovered in feces within seven days and only very little in urine. A complete recovery of radioactivity was not obtained in these studies within seven days. Five cows given a single dose of 15 mg/kg body weight of hexachlorophene excreted about half to two thirds in their feces within five days. Very little was excreted in urine and none in milk.

Metabolites were not identified and tissue levels were not determined in this study. The authors cited a study by Kok,[255] who did determine hexachlorophene in various organs of rats.

Carroll et al.[256] studied the absorption of C-14 labeled hexachlorophene on the tail skin of the rat. Hexachlorophene in a detergent solution was absorbed by the intact skin of the tail at the average rate of 1.7 $\mu g/cm^2$ of exposed surface area/hr. Immediately after the infliction of second degree burns, the absorption rate increased 2.5 times that of the normal skin surface. After 24 hr the absorption rate dropped to normal or subnormal levels. Immediately after traumatizing the tail, the absorption rate of hexachlorophene was even greater than following the infliction of a burn wound.

Manowitz and Johnston[257] found that hexachlorophene was deposited on the skin when used in various formulations and could be extracted from the skin by soaking the exposed areas (hands and forearms) in alcohol. The amount recovered increased in proportion to the amount applied. These authors were unable to confirm earlier investigations which suggested that the quantity of hexachlorophene retained by the skin reached a plateau level after a number of washings and remained relatively constant thereafter. Arms were washed and rinsed every 20 min. When extractions with alcohol were made on different arms after different numbers of washings, it was found that the amount of hexachlorophene recovered increased with the number of washings and no plateau was reached. A greater amount of hexachlorophene was also deposited on the skin with increasing length of time of a single washing. Since we now know that hexachlorophene is also absorbed from the intact skin and some of it is probably also lost from the surface of the skin, the time interval between the washings plays a role in the amount of hexachlorophene found to accumulate on the skin. Also of interest is that bathing water containing 4 mg/l of hexachlorophene deposited approximately the same amount of the chemical on the skin as a single washing with a 2% hexachlorophene soap.

Since hexachlorophene was used very extensively and often unknowingly by the general population, we investigated whether it was present in the blood of a few random samples of the general population. We found that the arithmetic mean concentration of hexachlorophene in adults was 0.028 ppm ($\mu g/g$) of whole blood of a total of 14 samples. In infants who were washed with hexachlorophene in the nursery the amount of hexachlorophene found in whole blood after the infants had anywhere from 2 to 14 washes averaged 0.109 ppm ($\mu g/g$ whole blood). Blood obtained from the cord at the time of birth contained an arithmetic mean of 0.022 ppm hexachlorophene.[258,259]

In the rats that were used in a reproduction study and were fed 100 ppm hexachlorophene in the diet for 258 days, the hexachlorophene blood levels averaged 1.21 ppm, while the blood levels of rats fed 500 ppm of hexachlorophene for 55 days who had severe symptoms of poisoning amounted to 8.5 ppm.[259,260] At the time we reported these blood levels, our recovery rate was only about 75%. All blood levels reported at that time were determined in whole blood. (It is assumed at the moment that blood levels are equivalent to half the amount of that found when plasma is analyzed.) A study undertaken at the request of Sterling Laboratories[132] illustrated that newborn rhesus monkeys following total body bathing with a 3% detergent solution for 1 week had blood levels of about 1.5μg/ml (ppm). In subsequent months a plateau effect was observed and at 90 days the blood level was equivalent to 1.1 μg/ml. These monkeys showed status spongiosus of the white matter of the brain. this indicates that at least in the monkey, blood levels of about 1.5 μg/ml whole blood were consistent with fluid accumulation in the myelin sheath of the central nervous system.

Lockhart[132] cited unpublished studies by Gluck, who found that hexachlorophene blood levels in term infants after washing with a 3% hexachlorophene detergent solution were somewhat higher than those reported by us with a mean concentration of 0.345 ppm ($\mu g/g$ whole blood). This difference could have been due to a difference in washing procedure and a better recovery rate in the analytical method in Gluck's study, or both.

Premature infants apparently absorbed hexachlorophene more readily. In 15 premature infants that were washed with a 3% hexachlorophene detergent solution, the average blood concentration amounted to 0.475 ppm ($\mu g/g$).[261] Abbott et al.[262] using a different washing regimen found a mean hexachlorophene blood concentration 12 to 18 hr after the initial wash of 0.154 ppm ($\mu g/g$

whole blood) and on the 7 to 9th day of life, 24 to 48 hr after the second or third application of hexachlorophene, the mean blood level amounted to 0.287 ppm (μg/g). The amount found in cord blood of 16 infants was 0.013 ppm (μg/g). When hexachlorophene was applied only on the day of delivery, the hexachlorophene blood levels gradually decreased from a mean of 0.145 ppm on the first day to a mean of 0.057 ppm on the ninth day, following an increase to 0.194 ppm on the fourth day. The authors suggested that premature infants may absorb more hexachlorophene through their skin since in one infant 29 weeks of gestation and weighing 1.42 kg the blood level was 1.17 ppm 24 hr after the initial wash and 0.655 ppm at 48 hours. Alder et al.[263] reported mean hexachlorophene blood levels of 0.18 ppm in infants who were dusted with a powder containing 0.33% hexachlorophene.

Adults using hexachlorophene preparations also absorbed it via the skin.[132] The blood levels obtained in adults that exposed their total body surface to a 3% hexachlorophene preparation twice daily for 60 days, 5 min each time, showed blood levels of approximately 0.68 ppm (μg/ml whole blood) with a range of 0.25 to 1.08 ppm (μg/ml whole blood). If only hand washing was employed for a period of 28 days, 4 times daily, a mean blood level of 0.07 ppm was reached and in a face hand washing study the mean blood level after 28 days was 0.196 μg/ml.

Blood levels obtained in a patient with burns who died between 24 and 48 hr after the last hexachlorophene application were 2.2 ppm (μg/g whole blood). The blood levels that have been reported all indicate that following dermal hexachlorophene exposure the chemical can be found in blood, and from information through animal studies as well as some human experience, levels of approximately 1.5 to 2 ppm (μg/g) in whole blood in humans are presently considered to represent toxic levels if the analysis for blood was performed with the methods mentioned elsewhere in this article.

Probably a better way of assessing a toxic dose would be to measure the amount of hexachlorophene in the brain, but for obvious reasons that is not possible. Female rats fed hexachlorophene at the rate of 500 ppm (25 mg/kg body weight/day) for 55 days with very definite lesions had about 4 ppm hexachlorophene in their brain and 3.91 ppm in the liver. Squirrel monkeys given 5 mg/kg body weight/day of hexachlorophene for 38 days showed a mean hexachlorophene concentration in the brain of 0.38 ppm and in adipose tissue the hexachlorophene concentration was 0.94 ppm.[264] The recovery rate in this study was only 75%. These squirrel monkeys had no overt clinical signs of toxicity, but showed mild status spongiosus of the white matter of the brain. Electroencephalographic changes had also been observed in these primates. Ulsamer et al.[265] found hexachlorophene brain levels of about 1 ppm in newborn rats with signs of central nervous system toxicity. In 1 suspected case of human hexachlorophene poisoning in a child, the hexachlorophene brain level was about 2.2 ppm.[261] Adult rats dying after the administration of a single dose of hexachlorophene showed concentrations of 6 to 9 ppm of hexachlorophene in the brain.[266] Some variation of the concentration of hexachlorophene in the brain which can be related to neurotoxic symptoms must be expected in different species and perhaps also with age and sex. Whether the fluid accumulation which may occur in the myelin sheaths after repeated exposure to hexachlorophene would alter hexachlorophene brain levels has thus far not been reported.

With present methods, we are probably only determining free hexachlorophene while the conjugated portion remains undetected. The toxicity of hexachlorophene will vary with the ability of the organism to conjugate, metabolize, and excrete this material.

We found in rats that hexachlorophene is excreted in milk of dams fed hexachlorophene at the rate of 20 and 100 ppm in the diet (2.3-1.1 mg/kg/day and 11.8-5.5 mg/kg/day, respectively, for 241 days). Electron capture, gas-liquid chromatographic determinations revealed levels of 0.07 and 0.33 ppm in the milk. Since hexachlorophene did not seem to accumulate to a great extent similar values would probably have been obtained, had the levels in milk in these animals been measured earlier. Although we now have some information on the absorption and distribution as well as excretion of hexachlorophene, the possible storage of the material as well as its metabolism needs further investigation. Since hexachlorophene was found in cord blood of infants, it can be assumed that transplacental passage also occurred.

Morphologic Effect on the Liver

Some of the chlorinated naphthalenes, chlori-

nated dibenzodioxins, chlorinated dibenzofurans, the chlorinated biphenyls, as well as the chlorinated terphenyls have an effect on the liver in various species, including humans. Bennett et al.[267] compared the toxicity of differently chlorinated naphthalenes and a biphenyl that contained 65% chlorine. Tri-chloronaphthalene apparently was less hepatotoxic than the higher chlorinated compounds and the chlorinated diphenyl was the most toxic of all. The studies were done in rats and the exposure was by feeding as well as inhalation. The findings described in the liver consisted of liver necrosis, fat accumulation, and the presence of hyaline bodies in the cytoplasm of the liver cells. A marked deposit of finely divided granular yellow or yellow-brown pigment was observed in Kupffer cells, particularly in those animals that had been exposed to the chlorinated biphenyls. This pigment apparently did not stain characteristically for either hemosiderin or hemofuchsin. Of course, all livers were enlarged macroscopically; in addition, these authors described proliferative changes in occasional bile ducts, particularly after feeding mixtures of chlorinated naphthalenes and chlorinated biphenyls. The number of bile ducts was increased in certain areas and the epithelial cells lining the bile ducts showed mitotic figures.

In inhalation studies with low concentrations of PCBs which consisted of either a 16-hr daily exposure to average concentrations of 0.57 mg/m^3, or to 8 hr daily with an average concentration of 0.93 mg/m^3, neither group of rats appeared ill. The periods of exposure varied from 37 to 143 days. When the animals were sacrificed the livers were pale or slightly yellow and somewhat mottled. Microscopic examination of the livers revealed similar changes as had been observed after feeding the material, and an increased vacuolation of the liver cells. Microscopic evidence of recovery was not observed in the livers of rats that had been exposed to the chlorinated diphenyl for a total of 105 days and were removed from exposure for 2 months.

Similar findings were made by Miller[268] who studied the effect of a PCB which contained approximately 42% chlorine. This author observed varying degrees of liver damage after subcutaneous injection as well as oral ingestion of the toxic substance in guinea pigs, rats, and rabbits. The liver damage consisted of fatty degeneration and atrophy of the central lobular cells. In the rat, hyaline bodies within the cytoplasm of the liver cells were also noted. Most of the liver damage was found in the guinea pig, less in the rabbit, and least in the rat.

Nishizumi,[269] who studied the effects of a Japanese biphenyl Kaneclor 400 with 40% chlorine in monkeys and mice, described enlargement of liver, fatty changes, granular cytoplasm of the hepatocytes, increases in the size of Kupffer cells and hepatocytes, and a brown pigment in some of these cells. He also studied the livers under the electron microscope and found an increase in smooth endoplasmic reticulum, variation in the appearance of mitochondria, and an increase in the number of microbodies. In addition, "myelin figures" were observed in the cytoplasm of hepatocytes.

Vos and Koeman[95] observed liver necrosis in chickens that were dosed with European PCBs, while a Monsanto product with 60% chlorine did not produce this effect. Increased amounts of iron were demonstrated with Perl's iron stain in the livers of all chickens exposed to the European as well as the American PCB sample. Vos and Beems[96] studied the PCB-induced lesions in the liver of rabbits, following dermal exposure to Aroclor 1260, Phenoclor, and Clophen. The microscopic changes observed in the liver included fatty degeneration, focal necrosis, centrolobular liver cell atrophy, ceroid pigment in Kupffer cells, periportal fibrosis, and cytoplasmic hyaline degeneration.

The feeding of Aroclor 5460, a chlorinated terphenyl, Aroclor 1254, or a chlorinated dibenzo-p-dioxin, not further specified in the article, led to liver hypertrophy of Sprague-Dawley rats within eight days to three weeks. Ultrastructural alterations were similar in the three groups and consisted of numerous multilayered, concentric membrane arrays and proliferated smooth endoplasmic reticulum. In many instances these membrane arrays surrounded lipid droplets.[270] Rabbits given 300 mg of Aroclor 1242 and 1254 once a week for 14 weeks developed enlarged livers and the ones given Aroclor 1254 showed midzonal necrosis of the hepatic lobules. The livers of rabbits fed Aroclor 1221 in a similar fashion did not show any histologic changes.[271]

We conducted studies to compare the toxicity of Aroclor 1254 and Aroclor 1260 in Sherman strain rats. The acute effect of these two mixtures on the liver was negligible. If rats were given a

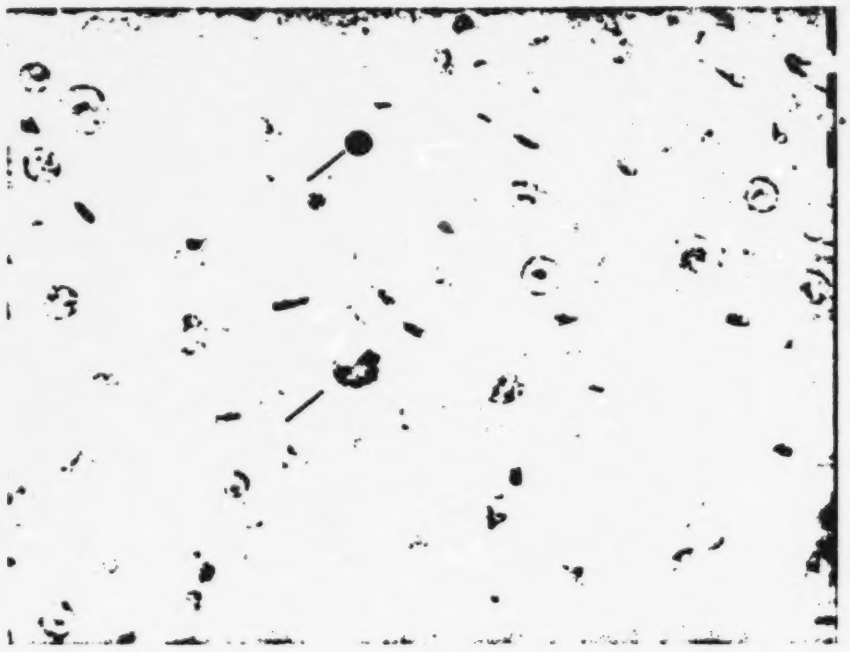

FIGURE 3. Section of liver of a rat fed Aroclor 1260 for 90 days. This figure illustrates a mitotic figure and a hyperchromatic nucleus (arrows) H & E X 500.

single dose of 10,000 mg/kg in peanut oil by stomach tube, and were sacrificed the next day, the liver was essentially normal microscopically. Doses of 1,000 ppm (72 mg/kg) of Aroclor 1260 for 3 months produced only an increase in mitotic figures (Figure 3) and enlarged hepatocytes. When groups of rats were fed dietary levels of 20, 100, 500, and 1,000 ppm of Aroclor 1260 and 20, 100, and 500 ppm Aroclor 1254 for 8 months, the liver changes were pronounced.[272] The hepatocytes were enlarged in many of the rats of the experimental groups and lipid accumulation, foamy cytoplasm, and a brown pigment which stained partially positive for hemosiderin were observed in the livers. The pigment was primarily observed in macrophages and Kupffer cells. Inclusions (Figure 4) that stained slightly more eosinophilic than the surrounding cytoplasm were present in the cells. These inclusions within the cytoplasm have been described for many compounds such as mirex[273] (dodecachloro-octahydro-1,3,4-metheno-2H-cyclobuta[cd]pentalene), DDT[274], 1,1,1-(trichloro-2,2-bis(p-chlorophenyl) ethane dieldrin[275], 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a-5,6,7,7,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene, and piperonyl butoxide[274] (α-(2(-butoxyethoxy)ethoxy)-4,5-methylenedioxy-2-propyltoluene). They are identical to the so-called hyaline bodies that were described in the earlier literature by Bennett et al.[267] and Miller.[268]

Aside from these general changes, extensive, grayish-white, firm, glistening areas were noticed in the livers of a number of the experimental animals, particularly at the high dietary levels. Microscopic examination of these grayish-white areas showed that the hepatic parenchyma had been replaced by glandular pale staining epithilial cells that formed ducts and were surrounded by proliferating fibrous tissue (Figure 5). Larger lesions often had extensive fibrosis and also contained collagen and the ducts that were formed by epithilial pale staining cells were markedly dilated and contained necrotic debris or mucus. The lesion has been classified as adenofibrosis for the time being. It has been described in detail by Edwards and White[276] who observed it in rats that were fed butter yellow (p-dimethylamino-azobenzene). These lesions enlarge peripherally by the formation of new glandular structures. Hepatic

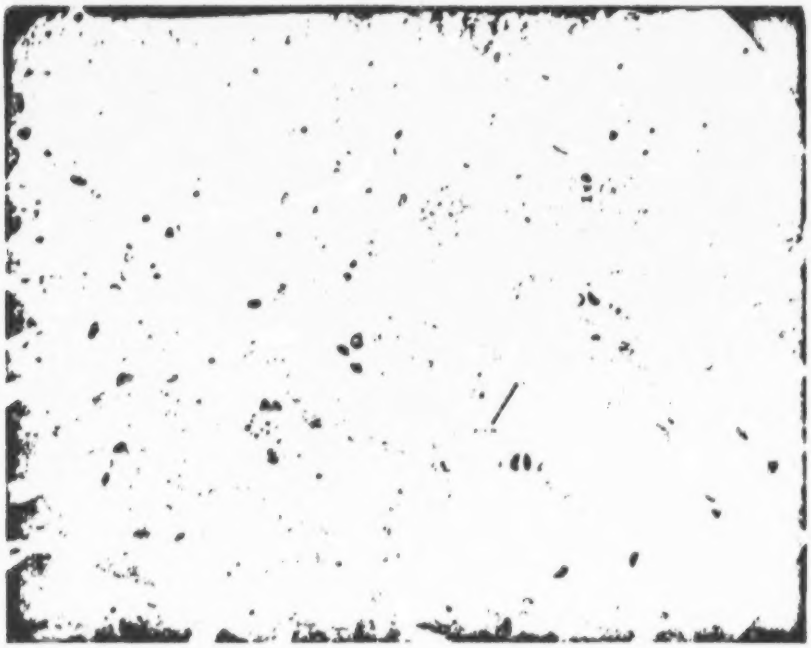

FIGURE 4. Section of liver from rat fed Aroclor 1254. A cluster of inclusions is present in the cytoplasm of one hepatocyte (arrow). Usually individual cells contain only one of these formations at a time. Toluidine blue stain of maraglass embedded material X 300.

tissue may be trapped between the glands of the peripheral area of adenofibrosis. The older central parts of the lesions sometimes contain pigmented avascular collagenized stroma with atrophic epithelial cells lining dilated ducts that appear atrophic and dead. The glands of actively growing adenofibrosis show atypical epithelium with large pleomorphic nuclei. The epithelium can occasionally become stratified. Parts of the lesion may be quite extensive, while in addition, in many areas of the same liver small foci of adenofibrosis exist simultaneously in close relationship to portal areas. It has therefore been assumed that these lesions arise from bile ducts, but they can also be primarily confined to the region of the hepatic veins. The absence of bile ducts around hepatic veins led to the conclusion[277] that a precursor, other than bile ducts, existed from which adenofibrosis developed. However some investigators of the lesions have assumed that it represented an atypical bile duct proliferation, also referred to as cholangiofibrosis.[278] In addition to this lesion we also observed small clusters of glandular cells within areas of adenofibrosis as well as adjacent to blood vessels that were surrounded by relatively normal appearing hepatic parenchyma with staining characteristics of salivary gland tissue. These clusters of cells very closely resembled pancreatic tissue.[279] The distinction between adenofibrosis and adenocarcinoma is difficult. The glands are quite atypical in adenofibrosis but it may be helpful to remember that in adenofibrosis, in contrast to adenocarcinoma, the irregularity observed is limited and duplicated with considerable exactness from lesion to lesion. Mucus is always present in adenofibrosis, but not always in adenocarcinoma. The stroma of adenofibrosis is concentrically arranged, young and cellular at the periphery and old and hyalinized towards the center of the lesion.[277] Of course a liver showing adenofibrosis may also contain carcinomatous lesions. So far conclusive evidence is lacking in the literature that adenofibrosis gives rise to carcinomas. However, it has been found to coexist with carcinoma of the liver in rats and some investigators feel that the lesion is in fact a precursor of cancer.[280] Choline deficient rats apparently, in addition to other changes in the liver, also develop

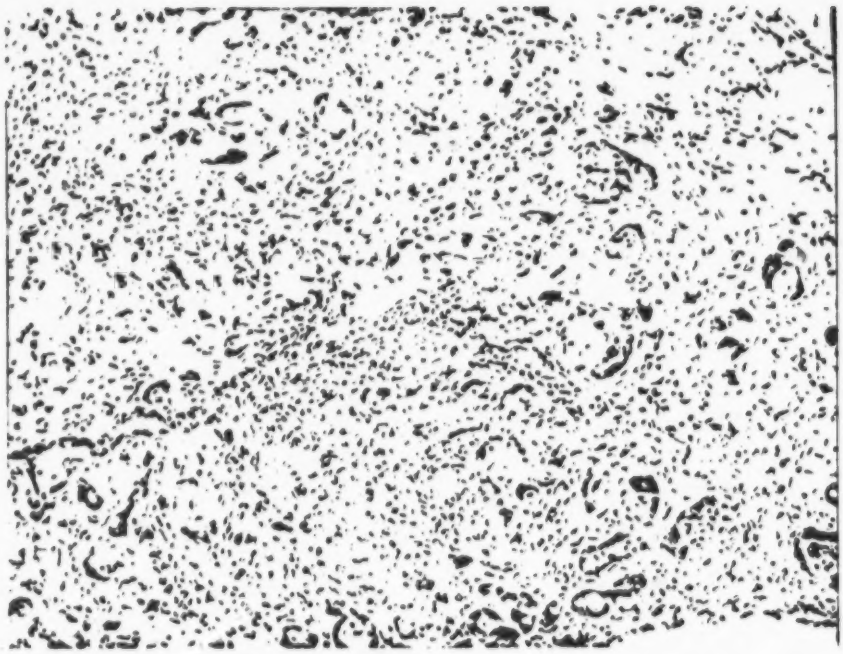

FIGURE 5. Section illustrates an area of adenofibrosis of the liver of a rat fed Aroclor 1254. Note the epithelial cells forming small ducts. The ducts contain cellular debris and are surrounded by fibrosis which can become quite pronounced. H & E X 125.

adenofibrosis.[281] However, these rats were fed peanut meal and soymeal and therefore the possibility exists that the experimental diet was contaminated with a mycotoxin. Bennet et al.[267] also described the lesion (adenofibrosis) in rats when a mixture of chlorinated naphthalenes and chlorodiphenyls was fed to them. A critical review of the histopathogenesis of this lesion was made by Stewart and Snell.[277]

Ultrastructural changes of the livers of animals exposed to the Aroclor consisted of an increase in smooth endoplasmic reticulum and atypical mitochondria. Many lipid vacuoles were observed, particularly at the higher dietary levels. They were at times surrounded by concentrically arranged membranes. The "inclusions" or "hyaline" bodies observed in the cytoplasm with the light microscope correspond to these formations when they are examined under the electron microscope. Their ultrastructural appearance has led to their being referred to as "finger prints" (Figure 6). The epithelial component of the adenofibrosis outlined consisted of cuboidal or columnar cells with the free surface lined by microvilli, granular cytoplasm with many ribosomes, and a few endoplasmic membranes. Some of the cells contained a great deal of mucus and resembled goblet cells. Tonofilaments and terminal bars were also observed. These various observations suggested that the epithelial component of adenofibrosis had all the morphological characteristics of bile duct epithelium.

Chickens with chick edema disease and cattle with the X-disease, already mentioned, usually showed changes in the liver which consisted predominantly of liver cell necrosis and fat accumulation. The hepatotoxic effect of chlorinated naphthalenes has also been described in rats.[267]

In a number of reports on the occupational occurrence of chloracne in workers that were exposed to chlorinated naphthalenes, chlorinated diphenyls, or 2,4,5-T, and technical trichlorophenol, reference is made to the hepatotoxic effect of these compounds. This hepatotoxic effect can manifest itself only in a decreased tolerance for alcohol; it may also develop into hepatic deficiency with acute yellow atrophy and jaundice. Apparently the effect on the liver was

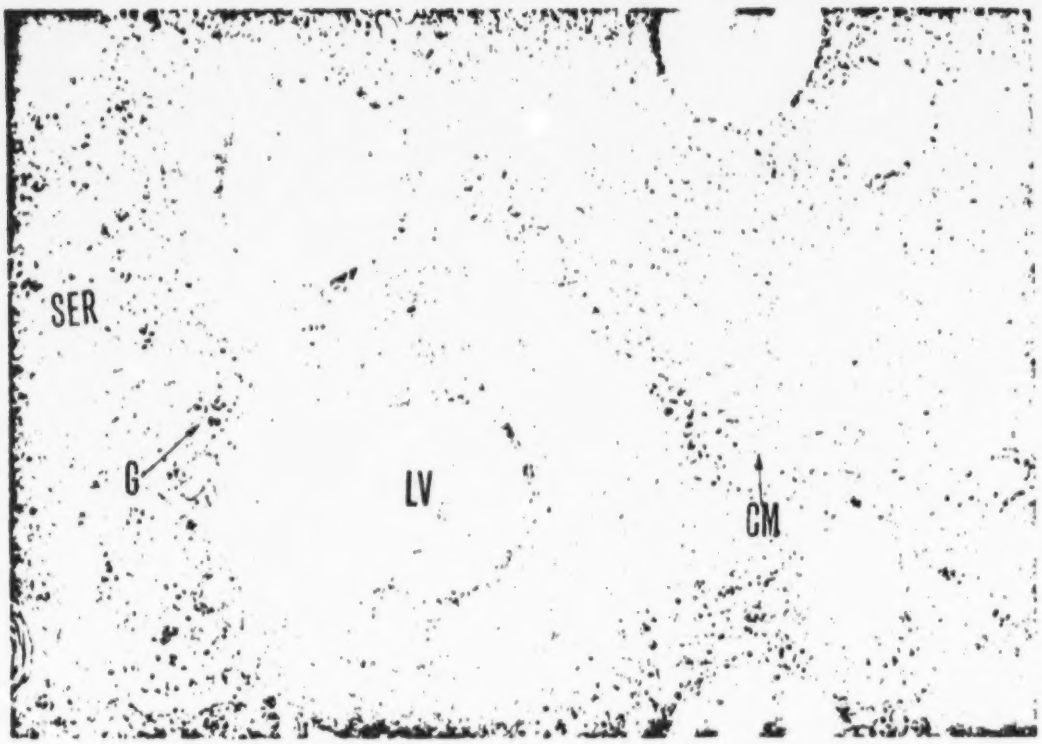

FIGURE 6. Electron micrograph of a portion of the cytoplasm of a hepatocyte. The section shows concentrically arranged arrays of membranes which surround vacuoles containing lipid. Lead citrate, uranyl acetate X 34,200.

particularly bad following exposure to halo-waxes, which are chlorinated naphthalenes.[282]

Cotter[283] reported seven cases of pentachlorinated naphthalene poisoning in workers who were engaged in manufacturing wire cable during World War II for the navy. Four of the workers developed jaundice and two died. Microscopic examination of the liver of the two workers that died showed complete loss of liver cells in some areas, the centrolobular areas were hemorrhagic, and prominent bile duct proliferation was seen in the periphery.

Another group of compounds that have at least some representatives that cause a hepatotoxic effect are the chlorinated dibenzodioxins and chlorinated dibenzofurans.

Single oral doses of 1 to 10 μg/kg *2,3,7,8-tetrachlorodibenzodioxin* given to rabbits produced a hepatotoxic effect. At the higher dosage level the animals died and the lower doses caused serious liver damage.[108] Schulz[107] had reported earlier that single oral doses of 20-50 μg/kg body weight resulted in fatal liver necrosis in rabbits.

Weanling rabbits were treated with 2,3,7,8-tetrachlorodibenzo-p-dioxin by applying a dose of 1.67 μg daily to the inner aspect of both ears for three days. The total dose given was 7.05 μg/kg body weight. When these rabbits were sacrificed 18 days following the last application of the dioxin, their livers were significantly larger than those of the controls. Microscopic examination of the livers showed enlarged hepatocytes. Some of the hepatocytes were multinucleated. The cytoplasm was foamy or vacuolated. A light brown pigment in some liver cells as well as Kupffer cells was also observed. Inclusions were seen in the cytoplasm in some liver cells, slight interstitial fibrosis was present, and two of three rabbit livers examined showed foci of necrosis that were surrounded by fibrosis.[284]

Studies with "toxic fat," the cause of chick edema disease, showed that it had a necrotizing

effect on the liver in chickens. As we now know, the toxic agents responsible for chick edema disease are chlorinated dibenzo-p-dioxins. Toxic fats also altered liver morphology in Macacca mulatta monkeys.[285] Hepatocytes were enlarged, multinucleated and focal necrosis in the centrolobular zone was observed. Many liver cells were vacuolated and stained positive for neutral fat.

In our studies of rats given *hexachlorophene*, we did not observe any liver pathology with the light microscope and no change with the electron microscope at the dosage levels that we employed.[118] Thorpe[121] on the other hand, observed periportal fatty changes in livers of sheep given three or four doses of 50 mg/kg hexachlorophene and histochemical changes were also observed in the hepatocytes. Pugh and Crowley[286] also observed hepatotoxicity in sheep after giving them hexachlorophene.

Of the other germicides discussed in this article, only triclosan (Irgasan DP-300) (2,4,4-trichloro-2′-hydroxydiphenyl ether) has been reported to have a toxic effect on the liver at a dose of 125 mg/kg and enlarged the liver of rats at a dose of 25 mg/kg, according to the summary minutes of the OTC Panel, U.S. Food and Drug Administration. It was not stated whether these were single or repeated doses. Oral administration of triclosan to beagle dogs produced jaundice and severe liver damage was observed. The effect that these compounds have on the liver will have to be studied further.

The accumulation of fat in the liver indicates that either fat transport or fat metabolism is impaired; if the insult to the liver is removed, the liver gradually recovers unless the lesion has become irreversible. The increase in smooth endoplasmic reticulum as well as the "inclusions" will also disappear after a certain amount of time if exposure to the material is discontinued.[287] The significance of the increase in smooth endoplasmic reticulum and the hyaline bodies with a concomitant rise in liver microsomal enzyme activity is presently not understood. These changes are considered adaptive and beneficial, but in addition to metabolizing unwanted chemicals at a higher rate, the stimulated liver also metabolizes some medications more rapidly,[288] as well as substances produced by the body such as steroids. Whether the inclusions within the cytoplasm represent the so-called inactive smooth endoplasmic reticulum which is deposited in the form of arrays of membranes within the cell is not known. The fact that smooth endoplasmic reticulum may become hypoactive was first postulated by Hutterer et al.[289]

Fibrosis of the liver as well as necrosis and the adenofibrosis observed after PCB exposure in rats should definitely be considered a very serious lesion. Usually adenofibrosis occurs concomitantly with hepatomas or hepatocarcinomas in rodent livers. A recent publication by Kimura and Baba[289a] showed that hepatomas can be induced in rats with Kanechlor 400. Chronic feeding studies may show that other PCBs will also induce hepatomas in rodents.

Neurotoxicity

In some of the reports on chloracne and also in the Yusho incident, peripheral neuropathy is mentioned as well as psychic alterations[1,290] as part of the symptomatology of poisoning, but in most reports not much emphasis was put on it. In the Yusho epidemic Murai and Kuroiwa[290] performed more detailed examinations in 21 cases admitted consecutively to Kuyushu University Hospital in the northern part of Japan. Seven of the patients complained of numbness or pain in the peripheral portion of their extremities and in five cases, hypoesthesia, hypalgesia, and thermohypesthesia were noted. Slowing as well as reduction of the sensory nerve conduction velocity in the radial and sural nerve was observed in a number of these patients. Headache and peripheral nervous system symptoms were also reported in a poisoning episode which occurred in a Finnish company where paper was impregnated with biphenyl.[92]

The only chemical of the group of polychlorinated, polycyclic compounds discussed in this report that has a predominant central neurotoxic effect in animals as well as humans is hexachlorophene. Adult female rats that were fed 500 ppm hexachlorophene in the diet for 14 weeks (about 30 to 18 mg/kg body weight/day) developed leg weakness after 12 to 19 days of exposure. This leg weakness which was only evident in the hindlegs progressed to paralysis within 3 to 5 weeks.[291] At autopsy the brains of the exposed rats were enlarged; they weighed an average of 2.6 g while the control brains weighed an average of 1.99 g. Microscopic examination of the brains revealed normal gray matter throughout the brain and the spinal cord in the few instances that the spinal

FIGURE 7. Electron micrograph from the white matter of the brain of a rat, illustrating status spongiosus. Note the large empty appearing vacuoles lined by myelin (MY = myelin, A = axon). Lead citrate, uranyl acetate X 34,200.

cord was examined. The white matter on the contrary seemed widened and showed many cystic spaces that appeared empty and were lined with strands of material that stained positive for myelin. The vacuoles were very numerous and varied greatly in size giving the tissue a spongy appearance. The brains in these studies were usually fixed in either Bouin's solution or buffered formalin for light microscopic study.

Brain tissue, particularly that of rodents, may normally show small vacuoles that are considered to represent an artefact.[292] These vacuoles are smaller, usually very uniform, and at times contain a homogeneous pink staining material. They are usually separated by a wider area of normal appearing white matter than the vacuoles in status spongiosus. Brains that show this artefact do not weigh significantly more than those that do not show it.•

Electron microscopic examination of brains of rats given hexachlorophene showed that the vacuolation in the white matter of the brain revealed large vacuoles lined by myelin. The axons that were present appeared usually to be quite normal; occasionally strands of myelin had separated and traversed the vacuoles. The vacuoles were otherwise empty except for some electron dense granular material (Figure 7). No other alterations were observed in the white matter. This lesion is not unique for hexachlorophene; other chemicals that have produced status spongiosus as we have called the lesion include triethyltin,[293] isoniazid,[294] the halogenated benzanilide, 2-acetoxy-4-chloro-3,5-diiodobenzanilide,[295] and rafoxanide,[296] which is the 3,5-diiodo-3'-chloro-4'-(p-chloro-phenoxy) salicylanilide. Triethyltin seemed to produce the lesion at a much lower dietary level and more rapidly than the other chemicals mentioned, particularly hexachlorophene.

In an additional study, after feeding rats 500 ppm hexachlorophene in the diet for 10 weeks and then discontinuing the exposure to hexachlorophene, function began to return in the hindquarters of paralyzed rats after 2 weeks and within

6 weeks the rats had almost completely recovered clinically. However, microscopic examination of the brains of these rats showed that a few cystic spaces were still present 12 weeks after the exposure to hexachlorophene had been discontinued. In weanling rats, but not in adult rats, the brain lesion and symptoms of paralysis could be produced with a single oral dose of 100 mg/kg of body weight given by stomach tube in peanut oil.[118] Part of the reason for being able to produce the brain lesion in the weanling rats with a single dose may be related to the fact that the weanling was able to survive a higher single dose than the adult rat.

Other species that have also shown paralysis and other neurological deficits after exposure to hexachlorophene are rabbits,[297] pigs,[298] and cats.[299] Sheep become blind according to Udall and Malone.[120] Hanig et al.[299] recorded the neurological deficits produced in cats in more detail. Early symptoms consisted of lassitude, weakness and ataxia of the hindlegs, impaired righting reflex, patellar hyperreflexia which later developed into hyporeflexia, urinary retention, and eventually complete flaccid paralysis. The cranial nerve functions remained intact except preterminally. In these cats an increase in cysternal cerebralspinal fluid pressure was observed.

In our first studies we continued exposure to hexachlorophene for a period of 14 weeks because we had designed the experiment to conduct an approximate 90-day feeding study. It is not necessary to feed rats for this length of time. As we determined later, microscopic changes can be observed in rats after a feeding period of two weeks when the animals were exposed to levels high enough to produce the lesion. Some vacuolation can already be observed 3 to 4 days after onset of exposure to hexachlorophene in some rats. When lower dietary levels were fed in a reproduction study,[118] the dietary level of 20 ppm (2.4 0.9 mg/kg body weight/day) had no effect on the brain. At the dietary level of 100 ppm (11.8 4.6 mg/kg body weight/day) focal areas of vacuolation of the white matter of the brain were observed in some of the rats, but they were never extensive. The rats in these lower dietary levels did not show any clinical neurological symptoms.

The brain lesion observed in rats was also described in monkeys following the exposure to hexachlorophene. Newborn monkeys washed with 3% hexachlorophene detergent solution developed a diffuse status spongiosus of the white matter of the brain.[300] Santolucito[264,301] was able to produce the vacuolation of the white matter, the so-called status spongiosus, by injecting squirrel monkeys daily subcutaneously with 5 mg hexachlorophene/kg of body weight for 37 days. When monkeys were *fed* hexachlorophene they did not develop the brain lesion but showed electroencephalographic changes which need further investigation. The monkeys that showed the vacuolation also demonstrated electroencephalographic changes. Sheep have apparently also shown status spongiosus of the white matter according to Hale and Reid.[302] Lampert et al.[303] were able to produce the lesion in mice as well as rats. These authors reported that the morphological changes were also observed in frozen sections of brains quenched in liquid nitrogen. Trypan blue injected intraperitoneally did not stain the brain, an indication that the blood brain barrier was intact as far as trypan blue was concerned.

The brain changes consistent with status spongiosus due to hexachlorophene exposure have also been described in humans, particularly children. Mullick[130] examined the brains of six human cases that died from hexachlorophene poisoning. The two adults in this group did not show vacuolation of the white matter of the brain. They had died less than 48 hr after exposure to hexachlorophene. The four children (two with congenital ichthyosis, two with burns) had at least three or more days of exposure to hexachlorophene baths in which a 3% hexachlorophene preparation was used. The final concentration of hexachlorophene in the bath water was not known. All four children showed status spongiosus of the white matter. The brain weight given in one of the cases, a 12-day-old child, was 425 g. The normal brain weight for a child that age is 382 g. Shuman et al.[304] in a retrospective blind study were able to demonstrate that the incidence of status spongiosus in the brain stem reticular formation in infants who had died from a variety of causes could be correlated to hexachlorophene exposure. The authors found 21 cases in a series of 250 autopsies of children. Of these cases, 18 were premature infants weighing less than 1,400 g and were of less than 30 weeks' gestation. All but 2 of the cases had 3 or more total washes with 3% hexachlorophene. In the group of cases that were studied were a number of stillborns who did not

show the lesion and of course had not had dermal hexachlorophene exposure. Shuman et al.[304] mentioned earlier cases of Letterer Siwe's disease in which they had observed status spongiosus of the white matter. Each of the children with Letterer Siwe's disease had had pronounced exposure to hexachlorophene because of the skin lesions which had been vigorously scrubbed with a detergent containing the chemical. The various observations show that, in patients with abnormal skin as well as the skin of the premature, hexachlorophene may be absorbed in sufficient amounts to cause status spongiosus. The fact that blood levels in premature infants washed with hexachlorophene tend to be higher than in mature infants also substantiates these findings.

Almost identical observations were made by Powell et al.[305] In premature infants weighing less than 1,400 g, 7 of 13 that had 4 or more exposures to hexachlorophene washes showed status spongiosus of the myelinated tracts of the brain stem. If this group was further subdivided according to the number of exposures, 6 of 9 infants that had 9 or more exposures to hexachlorophene washes showed the lesion, and 1 of 4 that was washed 4 to 8 times. Four stillborns and 13 infants washed 0 to 3 times with hexachlorophene and weighing less than 1,400 g did not show the spongy change. Electron microscopic examination showed that the spongy change observed in the premature infants was due to a split in the myelin sheaths.

In August of 1972 the press reported a number of deaths of infants in France that occurred because a baby powder to which hexachlorophene had accidentally been added at a concentration of 6% was used on the infants. The final death toll amounted to 41 infants and young children[306] (New York News, Dec. 13, 1972). Detailed accounts have not been published of this incident because of litigation problems. As we pointed out earlier, under acute toxicity, the higher the concentration of hexachlorophene is in a preparation applied to the skin, the more likely it is that skin damage will result. Skin damage also occurred in the French incident. In addition to this, the powder was probably used in many instances where some diaper rash already existed and was then well covered with a diaper, which may have contributed to increased absorption in this area. The brains and spinal cord of a few infants from the French poisoning outbreak that were examined microscopically showed status spongiosus of the white matter.[306]

These various accounts show that status spongiosus can also be produced in humans and probably represents a nonspecific reaction which can be elicited by a number of chemicals.

The lesion status spongiosus of the white matter as far as we now know seems to represent a specific or restricted type of brain edema where the fluid accumulation is confined to the inside of the myelin sheath and myelin is not actually damaged or at least breakdown products of myelin have not been observed. The term status spongiosus or spongy change has been used as a descriptive term and a spongy change of the gray matter may also occur in certain diseases. Status spongiosus together with other morphological changes can be observed in the brain in a variety of many etiologically different diseases and the reader is referred to Klatzo,[307] Ardonato and Lampert,[308] and a recent editorial in the British Medical Journal.[309] From clinically observed poisoning cases as well as animal experiments it is evident that the brain lesion is to a great extent reversible. However, in our own studies as well as those by Lampert et al.,[303] microscopic changes in rodents did not completely regress and the fact that sheep do not recover their eyesight after hexachlorophene poisoning[220] indicates that repair may not always be complete. No reasonable explanation exists at the moment to indicate why certain chemicals produce this very specific edema confined to certain structures of the central nervous system.

Gastrointestinal Lesions

Only two types of compounds under discussion have produced lesions in the gastrointestinal tract. We observed in our study with Sherman strain rats that high single oral doses of 3,000 mg/kg Aroclor 1254 and 1260 or more caused ulceration of the gastric and duodenal mucosa (Figure 8). Ulcers in the GI tract were not observed in the long-term feeding studies with 500 ppm or less. No other changes in the gastrointestinal tract were observed. Ringer et al.,[105] in their reproduction study with mink, observed that Arocior 1254 produced hemorrhage in the gastrointestinal tract of the offspring.

Allen and Norback[310] observed hyperplasia and dysplasia of the gastric mucosa in rhesus monkeys. Six of these rhesus monkeys were fed

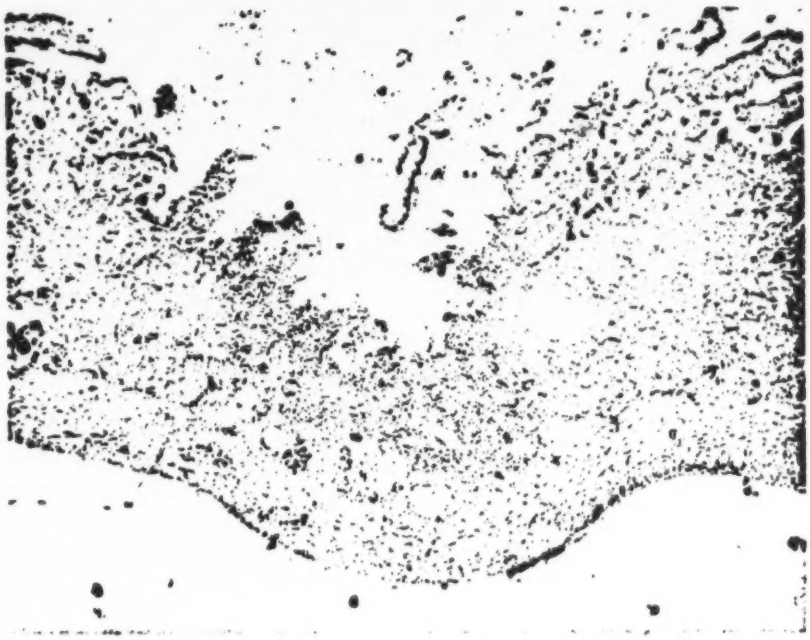

FIGURE 8. Section of the glandular portion of the stomach of a rat given a single dose of 3,000 mg/kg of Aroclor 1254. This figure illustrates the focal loss of mucosa and the infiltration by inflammatory round cells. H & E X 50.

300 ppm Aroclor 1248 and six were given 5,000 ppm Aroclor 5460, a polychlorinated triphenyl. The glandular formations in this lesion were highly atypical; penetration of the muscularis mucosae and invasion of the submucosa by the mucosal epithelium were observed. Allen and Carstens[285] reported an effect of toxic fat on the gastric mucosa in 18 of 27 monkeys, *Macaca mulatta*, which consisted of hypertrophy of the gastric mucosa and small gastric ulcers within the mucosa. The toxic fat used in these studies, as we now know, contained chlorinated dibenzodioxins.

It has also been observed that hemorrhaging occurred into the gastrointestinal tract of rat fetuses who were exposed to 2,3,7,8-tetrachlorodibenzo-o-dioxin in utero. It was not stated in the reports whether the gastrointestinal hemorrhages were caused by ulceration of the mucosa.[110,111] Ulceration of the glandular portion of the adult rat stomach was also observed.[113]

Patients with chloracne frequently complained of loss of appetite, nausea, vomiting, and abdominal pain, and Goldmann[311] described a worker with a follicular dermatitis who died six months after exposure to 2,3,7,8-tetrachlorodibenzodioxin. At autopsy a ruptured duodenal ulcer and a gastric ulcer were found. Whether any relationship existed between the ulcers and the exposure to the poisonous chemical was not mentioned in the paper.

It should be established if these gastric lesions also occur at lower dietary levels and whether the contamination of the chemicals with various chlorinated dibenzodioxins and furans is responsible for them. Valuable information might be obtained from retrospective studies of workers following occupational exposure. Whether the ulcers are caused by a direct effect of the chemical on the mucosa of the gastrointestinal tract or whether stress plays a primary role in their etiology similar to ulcers that may occur in burned patients for instance (Curling's ulcer) is not clear.

COMMENTS

The acute toxicity of compounds such as the chlorinated biphenyls, the chlorinated naphthalenes, and the chlorinated terphenyls is very low.

On the other hand, they are poorly broken down in the environment and the metabolism of the biphenyls with more than four chlorine atoms seems to be negligible. Because of these characteristics, the use, wherever possible, of chlorinated biphenyls that consist of mixtures of compounds with one to four chlorines has been proposed. Neither the chlorinated naphthalenes nor the chlorinated terphenyls have so far been shown to present a pollution problem.

Whether the various disease entities reviewed in this article are produced by all the different products mentioned in connection with them is not established with certainty. For instance, some of the compounds that produce chloracne or chick edema have been shown to be contaminated with various chlorinated dibenzodioxins and chlorinated dibenzofurans. The possibility remains that the compounds illustrated in Figure 1 are contaminated with these chemicals and the products themselves are not really responsible for the observed symptoms. On the other hand, a number of structurally unrelated compounds may produce the same disease entity.

Some of the chlorinated dibenzodioxins and the chlorinated dibenzofurans are highly toxic. The most toxic is 2,3,7,8-tetrachlorodibenzodioxins, while the dibenzodioxins without any chlorine and those with eight chlorines are much less toxic. The difference in toxicity of these compounds may be partly caused by the fact that they are very poorly dissolved and absorbed. Intravenous toxicity data are thus far not available on most of these compounds.

We also do not know at present if the porphyria which develops after prolonged exposure to a number of technical compounds such as hexachlorobenzene, chlorinated biphenyls, chlorinated dibenzodioxin, and 2,4,5-T is in many instances due to the contamination of these products with chlorinated dibenzodioxins; of course it may be possible that one or more chlorinated dibenzofurans also produce porphyria. It has also not been established whether all compounds that produce porphyria in animals will do so in humans as well.

In general, the exposure of the various population groups to chlorinated biphenyls and other cyclic chlorinated hydrocarbons is at too low a range to induce porphyria. Whether this can also be said for occupational exposure is not known. When production conditions and general hygiene of the workers are poor, porphyria cutanea tarda can apparently occur.[2] It is also not known if the combined effect of different porphyria inducing agents is cumulative. Whether the photosensitivity observed in humans with some of the germicides discussed in this paper is related to an effect on the porphyrin metabolism was not evident from the published literature.

So far the mechanism that causes chick edema, chloracne, X-disease, and liver disease has not been satisfactorily demonstrated. Chick edema disease has been thought to be caused by proliferation of the vascular endothelium. This might explain the fluid accumulation observed in diseased animals, but so far the sequence of events that led to this disease is not really clear and factors other than the embarrassment of the vascular system may play a role in its etiology such as a toxic myocarditis, decrease in serum proteins, and renal failure. More detailed study of the pathology of the various disease entities is needed for a better understanding of the development of the various lesions mentioned.

The chlorinated polycyclic hydrocarbons seem to alter the immune response of certain animal species and perhaps also of people. These very important findings need to be pursued further. Thus far it has not been established beyond doubt whether this is caused by a general debilitating effect on the animal of which atrophy of the lymphoendothelial system may be one manifestation, or whether this is a specific alteration, as we know it from the effects of radiation and exposure to certain alkylating agents. In this respect not enough attention has been paid to the function of the adrenal, and several of the observed symptoms in humans such as the hyperpigmentation suggest hypofunction of the adrenal cortex. Depending on the information obtained from these studies, a tolerance level of 5 ppm in some food products as set by the U.S. Food and Drug Administration may have to be revised particularly since no long-term low level feeding study has been reported in animals.

Many questions have recently been raised about the use of germicides in various toiletries, common soaps, etc. The objection to their use in the specific case of hexachlorophene is its neurotoxic effect, that can also occur in humans, particularly if the skin is defective in some way. Of course, limited localized exposure, such as handwashing or treatment of a small wound, will not produce neurotoxicity.

In infant nurseries in hospitals, one of the greatest problems is the constant threat of a staphylococcus infection of epidemic proportions. In order to prevent outbreaks of staphylococcus infections, a routine was developed in many hospitals in the U.S. and elsewhere in the early 1960's where infants were washed once daily with a 3% hexachlorophene detergent solution, and in addition the diaper area was washed several times a day. The detergent was subsequently rinsed off more or less vigorously, depending upon the philosophy of the different nurses. In some hospitals the detergent was directly applied to the infant, in others it was first diluted in water. Since the neurotoxic effect of hexachlorophene has become known, many hospitals have changed their policies on the use of hexachlorophene. Another drawback to this routine, in addition to a possible neurotoxic effect, is that hexachlorophene keeps not only the staphylococcus of certain phage types from growing on the skin, but reduces all Gram-positive bacteria,[312] which is highly undesirable and may lead to disease. Recently Evans et al.[313] have pointed out that a marked annual variation exists in the prevalence of several bacteria in the nares as well as the umbilical cord of newborns. A decline of staphylococcus aureus was observed in the fall; the prevalence of *Escherichia coli* and streptococcus also varied. Staphylococcus epidermis and Enterobacter organisms did not show this fluctuation. Any studies of efficacy undertaken in a clinical setting should take these normal fluctuations into account and it is possible that good general housekeeping practices are ultimately more effective in preventing bacterial disease in newborns than various germicides. Unfortunately, breaks in technic do occur which in many hospitals makes the use of germicides on infants desirable.

The information reviewed in this article shows that the toxic effects of the polycyclic polychlorinated compounds are complex. The mere knowledge of an LD_{50} value in an animal species does not give us a great deal of information about the toxicity of a compound. Toxicity or toxic effects are a relative concept and such household items as table salt, vitamins, pepper, and allyl propyl disulfide volatilizing from onions to name a few are also toxic. Nevertheless, we have to learn not to use chemicals indiscriminately, without knowing a great deal about them, particularly their long-range effects, when they are persistent and poorly metabolized. The indiscriminate use of these products and discharge into the environment invariably lead to their restriction or ban, which is accompanied by a great deal of emotionalism, distorted facts, and often great economic loss. Prudence, better judgment, and a critical approach to the usage of chemicals and drugs, particularly an evaluation of whether in specific situations they are necessary or not, may help us avoid these situations in the future. Particular attention should be paid to avoiding unnecessary exposure of children to chemicals that we know little about. Children may react differently from adults and as far as long-term exposure is concerned, they would be the most vulnerable.

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